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A SUMMARY OF ECOLOGICAL INVESTIGATIONS
AT EDGEWOOD ARSENAL, MARYLAND:
FISCAL YEAR 1970

by

F. Prescott Ward

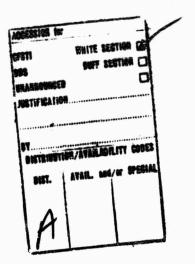
June 1971



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DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
Research Laboratories
Medical Research Laboratory
Edgewood Arsenal, Maryland 21010





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EDGEWOOD ARSENAL SPECIAL PUBLICATION

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A SUMMARY OF ECOLOGICAL INVESTIGATIONS AT EDGEWOOD ARSENAL, MARYLAND: FISCAL YEAR 1970

by

F. Prescott Ward

Veterinary Medicine Department

June 1971

Approved for public release; distribution unlimited.

Task 1B662710AD6302

DEPARTMENT OF THE ARMY EDGEWOOD ARSENAL Research Laboratories Medical Research Laboratory Edgewood Arsenal, Maryland 21010

FOREWORD

The work described in this report was authorized under Task 1B662710AD6302, Chemical Safety Investigations, Test Area Ecology. The experimental data are contained in notebooks MN-2277, MN-2331, MN-2349, MN-2357, 7698, 7797, and 8310. This work was started in August 1969 and has continued to the present time; this report summarizes investigations conducted in fiscal year 1970.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

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The author acknowledges the excellent cooperation of the following individuals and their assistance in the preparation of this manuscript: Mr. George L. Stem, Soil Conservation Service, US Department of Agriculture; Mr. Melvin Ginsburg, Baltimore County Department of Planning and Zoning; and MAJ David G. Fairchild, Mr. Everett A. Haight, Miss Dorothy Ward, Dr. Van M. Sim, MAJ Charles F. Ross, Mr. William Hyle, Mrs. Marion P. Royston, Mr. William V. Jeffries, Mr. Kenneth G. Hudler, Mrs. Jane Dierdorff, Mr. Marvin O. Sutor, Mr. Donald M. Bandel, personnel of the Maryland Army National Guard, and Mrs. Frances M. Bands, Edgewood Arsenal.

DIGEST

The ecological investigations described in this report were designed to measure the environmental impact, if any, of field tests of chemical warfure agents at Edgewood Arsenal, Maryland. This program was initiated in August 1969 in response to concern expressed by members of Congress and other public officials because of the existence of the testing activities.

This report contains appropriate background information concerning agent field testing at Edgewood Arsenal; the recommendations of the Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland, and Fort McClellan, Alabama; and physicochemical and climatological parameters of the test area. Summary reports of progress during fiscal year 1970 in various phases of the program are also presented.

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CONTRIBUTORS

- A. Dr. Seymour D. Silver, Director, Research Laboratories, Edgewood Arsenal, Maryland, initiated the Research Laboratories major program "Test Area Ecology" in July 1969.
- 1. COL Bruce S. Ott, "Test Area Ecology" Program Coordinator, July 1969 to March 1970.
- 2. Dr. Duane F. Ford, "Test Area Ecology" Program Coordinator, March 1970 to present.
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 - c. Mr. Edmund J. Owens, Toxicological Studies.
- (1) Mr. John T. Weimer, Investigations of Agent Toxicities to Estuarine Species.
 - d. Mr. Samuel Sass, Analytical Chemistry Studies.
- B. The Director of Technical Support was designated by the Commanding Officer, Edgewood Arsenal, as Action Officer for implementing the recommendations of the Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland, and Fort McClellan, Alabama.
- 1. Mr. Harold L. Gotoff, Chief, Field Evaluation Division, Technical Support Directorate, coordinated ecological studies with agent field-testing schedules.
 - a. Mr. George G. Norris, Test Area Sampling.

- b. Mr. Abraham Koblin, Analytical Chemistry Studies, in conjunction with Research Laboratories Analytical Chemistry Studies.
 - (1) Mr. Robert T. Howe, Agent Residue Investigations.

A SUMMARY OF ECOLOGICAL INVESTIGATIONS AT EDGEWOOD ARSENAL, MARYLAND: FISCAL YEAR 1970

INTRODUCTION.

This publication describes progress in ecological research conducted at Edgewood Arsenal, Maryland during fiscal year 1970 (FY70). The ecology program "Test Area Ecology," a Research Laboratories major program, was originally designed to measure the environmental impact, if any, of open-air testing of chemical warfare agents. This program now implements certain ecology-oriented recommendations made by the Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland, and Fort McClellan, Alabama, concerning the conduct of open-air chemical agent tests.

Chapter 1 is a brief history of chemical agent research, development, production, and testing at Edgewood Arsenal, Maryland. The impetus for formation of the advisory committee and the recommendations it made are included in chapter 2. Program planning to implement the ecology recommendations is discussed in chapter 3.

The committee's other recommendations concerned transporting agents, the weights and heights at which agents can be disseminated, and keeping the public and scientific community informed of results of the test program and safety practices. Because these recommendations influence our ecological research, progress toward their implementation during FY70 is presented in chapter 4.

The study area (test area) is described physically and ecologically in chapter 5. This section also contains a comprehensive review of agent trials on the test area during the preceding 5 fiscal years.

Chapters 6, 7, 8, and 9 contain summary reports of current investigations in the ecology program. Several studies have been completed, and manuscripts have been published (references cited) or are in preparation (indicated in text).

Pollution abatement, conservation and beautification of natural resources, and woodland and wildlife management efforts at Edgewood Arsenal are not within the purview of this program. These responsibilities are specifically assigned to, and are funded by, other elements of the installation. The results are recorded in other internal publications and reports.

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CHAPTER 1

BRIEF HISTORY OF EDGEWOOD ARSENAL, MARYLAND, AND CHEMICAL WARFARE AGENT TESTING

A. EARLY HISTORY.

Edgewood Arsenal is located on the western shore of the upper Chesapeake Bay on Gunpowder Neck peninsula in Harford County, Maryland, 21 miles northeast of Baltimore. Shortly after the acquisition of this land by the War Department in 1917, the installation became the center of chemical warfare agent research, development, and procurement for the United States Army.

In 1918 the reservation was established as a site for toxic gas filling plants. Its mission was further expanded to include the manufacture of toxic agents and the research and development of gas alarms, masks, protective clothing, and other devices destined to counter German gas attacks.

Between World War 1 and World War II, research and development of chemical agents continued, but at a reduced pace. World War II stimulated a major expansion of the installation to its present physical dimensions.

B. MISSION OF EDGEWOOD ARSENAL.

The mission of Edgewood Arsenal is to operate the commodity management center for chemical weapons and munitions, defensive systems, and related test and handling equipment. Commodity management—a single agency controlling the development of a specific item—begins in the idea stage and continues through research, development, test, evaluation, and finally to adoption or rejection.

C. AGENT FIELD TESTING.

Chemical items often require exhaustive field testing during various stages of their development, and open-air tests of limited quantities of agents have been conducted at Edgewood Arsenal for many years. In the early 1940's Gunpowder Neck was used jointly by Aberdeen Proving Ground (for ordnance field tests) and by the Chemical Warfare Board at Edgewood Arsenal (for chemical agent tests). In 1943 an increase in the need for agent field trials resulted in many scheduling conflicts for test areas, but Aberdeen Proving Ground tests usually received higher priority. The Chemical Warfare Board was therefore offered use of two government-owned tracts on the western shore of the Gunpowder River in Baltimore County: Carroll Island and Grace's Quarters. An extensive improvement program was initiated in March 1944; in 1951 Carroll Island became Edgewood Arsenal's major outdoor chemical agent test area.

The frequency of open-air tests is determined by requirements of the development program for new munitions and improvement of existing munitions, requirements of the medical research program, and requirements of the program for developing and engineering defense equipment. The quantities of agents released into the environment are relatively small during these tests. Field tests with large quantities of agents are conducted at Dugway Proving Ground, Utah; small quantities of chemical agents are also disseminated in conjunction with training exercises at the US Army Chemical Center and School, Fort McClellan, Alabama.

¹Chemical Warfare Board publication. Improvements. Carroll's Island. Gracv's Quarters. 1944. UNCLASSIFIED Report.

CHAPTER 2

THE BENNETT COMMITTEE

A. FORMATION.

In March 1968, the deaths of more than 6000 sheep in Skull Valley, Utah, caused public and Congressional concern and aroused objections to chemical agent testing.²

The Army agreed, in July 1969, to suspend open-air testing of lethal chemical agents at Edgewood Arsenal until safety procedures could be reviewed by an external committee of experts. (Lethal agent testing was discontinued on 14 July 1969 and had not been resumed as of 30 June 1970.) The impetus for this action was concern expressed by members of Congress and by other public officials because of the existence of the testing program. There had been no serious human illness related to testing at Edgewood Arsenal, no accident, no breach of safety, no evidence of damage to animals or plants, and no complaint by neighboring townspeople or local authorities.

On 21 July 1969, the Secretary of the Army established an Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland, and Fort McClellan, Alabama (referred to as the Bennett Committee in the remainder of this report). Committee members were:

Dr. Ivan L. Bennett, Jr., Chairman Director, New York University Medical Center Vice-President for Health Affairs, New York University

Dr. James B. DeWitt Division of Pesticides Registration Bureau of Sport Fisheries and Wildlife US Department of the Interior

Dr. Louis C. LaMotte, Jr.
Microbiology Branch, Laboratory Division
National Communicable Disease Center
US Public Health Service

Dr. Warren C. Shaw Crops Research Division Agricultural Research Service US Depart:nent of Agriculture

Dr. J. H. Wills
Institute of Experimental Pathology and Toxicology
Albany Medical College of Union University

LTC Robert F. Franz, Jr., Executive Secretary Director, Defense Development and Engineering Laboratories Edgewood Arsenal, Maryland

²Van Kampen, K. R., Shupe, J. L., Johnson, A. E., James, L. F., Smart, R. A., and Rasmussen, J. E. Effects of Nerve Gas Poisoning in Sheep in Skull Valley, Utah. J. Amer. Vet. Med. Ass. 156 (8), 1032-1035 (1970).

The Bennett Committee's charter stated:

The committee will review the procedures, talents, monitoring equipment, facilities and safety regulations used by Edgewood Arsenal and Fort McClellan in connection with chemical tests and detection and decontamination exercises. The purpose of the review will be to determine whether or not safety measures are adequate to insure that chemical tests and the detection and decontamination exercises do not constitute a hazard to humans and animals outside the housing and administration areas of the installation. The committee will make any recommendations deemed necessary to achieve this goal.

The committee niembers met at Edgewood Arsenal on 28 and 29 July 1969 for briefings about the testing program. They visited the Carroll Island test area and witnessed demonstrations of the testing procedure using munitions filled with a simulant agent. They consulted relevant technical reports, examined medical reports of the Toxic Exposure Aid Station, and reviewed the records of the natural resources conservation and beautification program.

B. RECOMMENDATIONS.

The report of the Bennett Committee³ was released in Septen ser 1969. It states in part:

The lethal testing program at Edgewood Arsenal during the past two decades has compiled an enviable record for safety. The testing procedures that have been evolved are clearly effective in minimizing danger to base personnel and civilians in adjacent areas. There is some hazard to the civilian community in the transportation by truck of agents and explosives to the test-grid area which has been the practice when ice prevents transportation by boat. There has been no obvious major detrimental effect of the testing program on the flora and fauna of the area although, until now, little or no systematic search for such effects has been carried out.

WE RECOMMEND

- 1. Resumption of the lethal testing program at Edgewood Arsenal under the existing safety procedures and precautions with the following modifications (which, although they tend to conform to existing practices, should be explicitly set down and observed):
- a. The more frequent use of mechanical and biological monitors beyond the periphery of the test-grid, including the downwind shoreline and over water;
- b. Explicit limitation of dissemination by spray or munition to heights below 75 feet.
- c. Explicit limitation of the weights of agents to be tested (the committee has not attempted to calculate these and, of course, any limitation must be defined in terms of meteorological conditions at the time of testing).
- 2. That the already planned program to determine effects upon foliage and soil be pursued vigorously and expanded to include observations on the

³Report of the Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland and Fort McClellan, Alabama. Department of the Army, Washington, D.C. September 1969. UNCLASSIFIED Report.

water-table at Carroll Island and marine life in the surrounding waters. The information should be collected at periodic intervals on a seasonal basis and compiled in a fashion that will make it easily available in an understandable and meaningful form.

- 3. That plans to fence off the periphery of the Carroll Island test area and to install filters on the vents of the controlled velocity test chamber (wind tunnel) on the Island be expedited.
- 4. That overland transport of agents and explosives to the lethal test grid area that involves leaving the reservation be minimized or, preferably, eliminated altogether.
- 5. That Edgewood authorities explore the possibility of some continuing formal arrangement with county, state or University-based conservation groups to conduct ecological surveys and an annual census of wildlife on the reservation.
- 6. That a conscious and continuing effort be made to give public visibility to the safety precautions used at Edgewood, particularly those aimed at protecting the public and preserving the environment. This should include regular briefings of appropriate local, county, and state officials, including health officials, state legislators, members of Congress, community groups, conservation organizations, and medical societies.

The Secretary of the Army approved the Bennett Committee Report and directed implementation of its recommendations in December 1969. Dr. Seymour D. Silver, Director of Research Laboratories, had initiated a major program entitled "Test Area Ecology" in August 1969. The objectives of this program fully satisfied several of the recommendations made by the Bennett Committee; thus, "Test Area Ecology" retained its identity after implementation was directed.

CHAPTER 3

PLANNING THE PROGRAM TO IMPLEMENT ECOLOGY RECOMMENDATIONS

A. RESPONSIBILITIES.

The Commanding Officer, Edgewood Arsenal, appointed the Director of Technical Support as action officer for implementing the six Bennett Committee recommendations. Responsibility for the ecologically oriented recommendations was delegated to Research Laboratories; COL Bruce S. Ott, VC, Chief, Veterinary Medicine Department, Medical Research Laboratory, was appointed program coordinator. COL Ott assigned the responsibility of preparing a prospectus for the ecology program to Dr. F. Prescott Ward; later, four participating scientists (Dr. Ward, Dr. Elmer G. Worthley, Edmund J. Owens, and Samuel Sass) were appointed by the program coordinator.

B. PROGRAM PLANNING.

Only Bennett Committee recommendations pertaining to ecology were considered in planning this program:

- 1. Use biological monitors (sentry animals) to augment mechanical sampling around the test grid.
 - 2. Continue research on the effects of chemical agents on foliage and soil.
- 3. Conduct investigations on marine life in waters surrounding Carroll Island and on the water table of the test area.
- 4. Conduct ecological surveys and wildlife censuses on the test area, preferably in conjunction with outside conservation agencies.

In a series of decision meetings, the participating scientists established research priorities and delineated the following basic goals.

- 1. Of the chemicals tested on Carroll sland, the lethal agents (anticholinesterases) had the most potential for causing the greatest damage to the surrounding biotic communities. Given highest priority was the development of capabilities that would facilitate rapid and significant investigations in the event of a suspected lethal-agent-related wildlife kill.
- 2. The choice of sentry and indicator species to augment mechanical sampling was governed by: sensitivity to the agents in question; accumulated baseline toxicological and biochemical data; seasonal availability; ease with which animals could be procured, transported, and confined; and cost. Sheep, white perch, starlings, and representatives of several trophic levels of the aquatic and terrestrial food chains were chosen.
- 3. Studies of movement and persistence of agent residues in the environment should be intensified.
- 4. The extent of ecological surveys and wildlife censuses would be governed primarily by available manpower, funds, and expertise. Efforts should be directed at many populations representing several trophic levels in the terrestrial and estuarine communities.

C. OBJECTIVES.

The following specific objectives, based on the priorities and broad goals established, were then formulated.

- 1. Establish baseline levels for the enzyme cholinesterase (ChE) in the blood, brain, and other tissues of selected species, including sentry species (sheep, starfings, and white perch) and several wildlife species of commercial or aesthetic value from the area (blue crabs, striped bass, diamondback terrapins, wild ducks, Canada geese, whistling swans, quail, bald eagles, cottontail rabbits, and white-tailed deer).
- 2. Conduct controlled laboratory exposures of selected wildlife species to known concentrations of agent to establish accurate toxicological parameters.
- 3. Measure ChE in sheep penned around the test grid, white perch caged around the periphery of Carroll Island, and random samples of starlings from the test area.
- 4. Establish laboratory aquatic food webs (algae, aquatic plants, zooplankton, large crustaceans, mosquito larvae, minnows) and determine sensitivities of these organisms to agents. Use this system to measure toxic effects of possibly contaminated water, soil, and vegetation from the test area.
- 5. Develop and refine analytical chemical procedures for measuring agent residues and degradation products in samples of air, water, soil, and vegetation from the test area.
- 6. Obtain samples of air, water, soil, and vegetation from the test area on a regular basis, and measure residues of agent and known degradation products or metabolites in each. Conduct controlled studies to determine the significance of environmental variables in the breakdown of chemical agents.
- 7. Continue studies on the role of plants in the uptake, concentration, transformation, and degradation of chemical agents.
- 8. Conduct basic ecological studies and establish continuing censusing procedures for representative plants and animals from the test area and surrounding waters, especially noting seasonal population fluctuations and interspecific population interactions.

Figure 1 is a sequence chart delineating these objectives administratively and chronologically.

D. MEETINGS WITH OUTSIDE AGENCIES.

After planning the ecology program, Edgewood Arsenal representatives briefed several state, federal, and university wildlife research agencies and invited criticisms of the program. Among these meetings were the following of special importance.

1. On 17 November 1969, Edgewood Arsenal scientists met with the Honorable J. Millard Tawes, Secretary, State of Maryland Department of Natural Resources. Also attending were Directors of the Departments of Chesapeake Bay Affairs, Forests and Parks, and Game and Inland Fish, and biologists assigned to these departments.

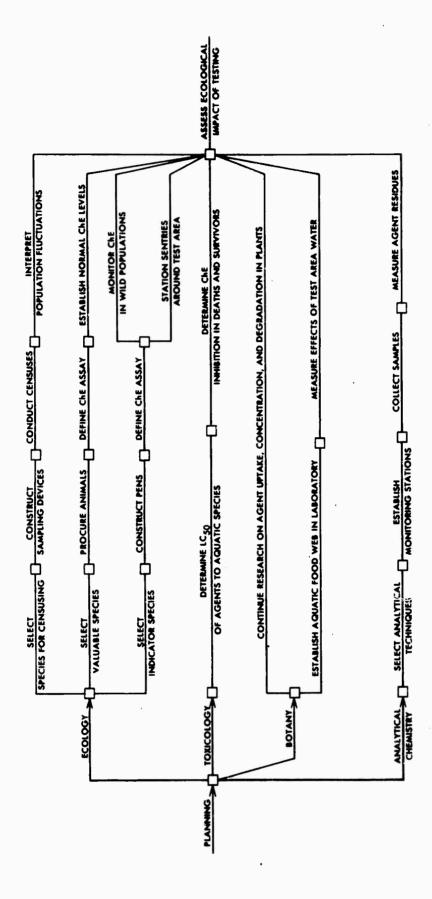


Figure 1. Sequence Chart With Responsibilities and Objectives of the Ecology Program Delineated

- 2. Dr. Eugene H. Dustman, Director, Patuxent Wildlife Research Center, US Department of the Interior, and several staff ecologists were briefed on 4 December 1969.
- 3. The Director of the University of Maryland Natural Resources Institute, Dr. Eugene Cronin, and several faculty members were briefed on the ecology program on 26 February 1970.
- 4. A meeting in Washington, D.C., on 10 April 1970 was attended by officials representing Edgewood Arsenal, the Office of the Secretary of the US Department of the Interior, the Bureau of Sport Fisheries and Wildlife, the Federal Water Pollution Control Administration, and the Bureau of Commercial Fisheries.

During these meetings, all representatives of outside agencies contacted expressed scientific approval of the design of the ecology program. Several suggestions that were made to strengthen our scientific approach were incorporated into the program. All agencies were eager to continue informal exchanges of information, ideas, and technical assistance.

CHAPTER 4

IMPLEMENTATION OF OTHER BENNETT COMMITTEE RECOMMENDATIONS

Responsibilities for implementing several recommendations made by the Bennett Committee that did not concern ecology were assigned to: Technical Support Directorate (recommendations 1, in part, and 4); Facilities Directorate (recommendation 3); and the Information Office (recommendation 6).*

1. All standard operating procedures (SOP's) for the conduct of open-air agent dissemination trials were revised to include the use of supplementary mechanical and biological monitors beyond the periphery of the test grid.

A small portable sampler was designed, constructed, and tested. The sampler can be mounted on a small floating device that can be anchored in position when placed in the water. Construction of additional equipment is now in progress, and sufficient samplers to facilitate the desired monitoring should be available in August 1970.

- 2. The explicit limitation of dissemination of agents by spray or munition to heights below 75 feet was incorporated into existing SOP's.
- 3. Each SOP has associated with it, in tabular form, downwind safety distances required for specific quantities of agent able to be released under prevailing meteorological conditions. Temperature gradient, as the indicator of the atmospheric stability factor, and wind speed, used in conjunction with the range limits of the testing site, explicitly limit the maximum amounts of agent that can be released safely. Safety distances are based on human no-effect levels (see chapter 5, Summary of Safety Procedures).
- 4. Interim fences of 5-strand barbed wire were constructed around Carroll Island and Grace's Quarters. Permanent fencing is budgeted in the Long Range MCA (Military Construction—Army) Program.
- 5. A scrubber decontamination system, in lieu of the filters recommended for the vents of the Carroll Island wind tunnel, is under procurement. The equipment, costing approximately \$25,000, should be delivered and installed by early in the 3d quarter of FY71.
- 6. Transport of agents and explosives to the Carroll Island and Grace's Quarters test sites has been entirely by boat within the bounds of the military reservation. Helicopters will be used to transport these materials, appropriately packaged, during the winter months when the Gunpowder River is frozen. No shipment over public highways or movement of chemical agents outside the confines of the reservation is anticipated.
- 7. With the exception of several press releases dealing with the ecology program (chapter 9), very little information was released to the public during FY70. Although Bennett Committee recommendations were ordered implemented by the Secretary of the Army, AR 360-41

^{*}Specific accomplishments in FY70 by all participants were reported in a letter, 25 June 1970, SMUEA-TSFE, from Acting Director of Technical Support to Commanding General, US Army Munitions Command, subject, "Implementation of the Recommendations of the Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland."

prohibits release of sensitive information and press visits without prior clearance. In addition, US Army Materiel Command message 192122Z Dec 69 further prevents implementation of recommendation 6, for it states that all information relating to chemical warfare will be released by the Office of the Assistant Secretary of Defense, Public Affairs.

The following is quoted from inclosure 6 of the letter cited earlier in this chapter.

It is strongly recommended that these stringent policies on release of CW/BR information be relaxed prior to approval of open-air testing. A reversal in current policy would enable the Edgewood Arsenal Information Office to respond immediately to press queries of local unclassified happenings without frustrating the press by providing "no comment" or delay answers pending approval from higher headquarters. Such a change in policy is urgently needed before unnouncing the resumption of testing if the credibility gap is to be overcome and favorable relations restored with the press. These deteriorated as a result of the Information Office at Edgewood Arsenal being prohibited from responding to queries or conducting a press briefing and tour when open-air testing was announced and halted in July 1969.

When and if open air testing is slated to resume, it is planned to fulfill Recommendation 6 by:

- a. A news release detailing actions taken to implement recommendations of Ad Hoc Committee.
- b. Release fact sheets and photos on all unclassified aspects of open air testing.
- c. Conduct briefing, tour and simulated test for state and local officials in advance of the announcement of the resumption of testing.
- d. Announce to the public plans for resumption of testing, stressing safety precautions and past safety record.
 - e. Conduct press briefing and simulated test.
- f. Schedule speakers to address local civic groups on need to conduct open air testing, safety, monitoring, environmental, ecological and conservation measures.

CHAPTER 5

THE CARROLL ISLAND/GRACE'S QUARTERS AGENT TESTING COMPLEX

A. GENERAL.

Carroll Island and Grace's Quarters are located on the upper Chesapeake Bay on the western shore of the Gunpowder River in Baltimore County, Maryland. The center of Test Grid 1 on Carroll Island is located at latitude 39°19′12″N., longitude 76°20′15″W.; the center of the Grace's Quarters open grid is located at latitude 39°20′45″N., longitude 76°20′28″W. The total land area of Carroll Island is 855 acres* (nearly 20% is forest) and of Grace's Quarters is 476 acres (more than 60% is forest).

Both areas are within the military reservation boundary of Edgewood Arsenal. They are used only for the outdoor testing of chemical warfare agents and munitions, defense systems, and related test and handling equipment. Carroll Island is the principal open-air test facility, and ecological investigations during FY70 have been confined almost entirely to this area.

When the tracts of land were offered to the Chemicai Warfare Board in 1943, results of surveys indicated that considerable improvements of both areas were necessary before they could be used as test sites. Portions of both had been leased as farm land, and the few existing roads were in poor condition; the areas were generally overgrown and were not acceptable as test sites.

A major improvement program was initiated in March 1944. Docks were built on both sites; during the next year men of the 100th Chemical Composite Company and German prisoners of war felled timber, drained and filled marshes, cleared proposed test fields of brush and marsh grass, built and surfaced many roads and bridges, and repaired or constructed several buildings on Carroll Island and Grace's Quarters.¹

Inspection of aerial photographs of Carroll Island taken shortly after this program was completed indicates that nearly all the timber on the eastern half of the island was felled. Thus the large woodlots that exist today in this area are less than 25 years old. Woodlots on other portions of the island, particularly the extreme western sectors, are considerably older.

In the testing history of Carroll Island, many surface fires have been intentionally started to clear brush or have been accidentally set by incendiary munitions. Extensive surface fires have involved the eastern half of the island several times in the last 15 years. During the same time period, several floods, usually associated with storm winds, are reported to have completely covered the eastern half of the island.

Each summer (records back to 1959) Edgewood Arsenal, including Gunpowder Neck, Carroll Island, and Grace's Quarters, is sprayed several times with an organophosphate insecticide for mosquito control. During the last 2 summers, aerial application of naled** (0.75 fl oz/acre) occurred usually four or five times per year at monthly intervals from late May to early September. Between 1959 and 1969 the spraying schedule was similar, but malathion was used. From July 1966 until August 1969, 95% malathion was applied at a rate of 3 fl oz/acre. In June 1966, 57% malathion in fuel oil was used: prior to that date, 15% malathion in fuel oil was applied (total insecticide sprayed was approximately 0.5 lb/acre by both methods).

^{*}Acreages are approximate with an inherent error of ±5%; areas were planimetered by William Hyle, Facilities Directorate, Edgewood Arsenal, in July 1962

^{**}Dibrom^R 14, Chevron Chemical Company, Ortho Division, San Francisco, California.

A herbicide* has been used on Carroll Island once or twice a year to control foliage in limited areas around Test Grid 1 and the magazine area. This application is necessary in the inner grid area to facilitate decontamination and to insure that vegetation does not interfere with dispersal and sampling of agent clouds. Weeds are controlled in the magazine area to prevent a fire hazard.

No significant wildlife management procedures have been employed on Carroll Island since chemical agent testing began in the area more than 20 years ago. There have been no population status studies, no control of predators, no stocking of game species, and no supplemental feedings or habitat improvements. Crushed oyster shells were used extensively as a roadbed material (this available calcium-phosphorus supplement may be responsible for the reproductively successful population of ring-necked pheasants on the island; this species is rare in other parts of Harford and Baltimore Counties). No hunting is permitted except for limited waterfowl gunning, in season, on selected areas of the shoreline of Carroll Island.

B. PHYSICAL DESCRIPTION.

Carroll Island is composed of Atlantic Coastal Plain soils, i.e., soils that have developed in sediments and have no underlying rock (bedrock) within significant depths.** The topography of Carroll Island, as charted in a 1962 survey, is shown on the map in figure 2. Soils of Carroll Island include 14 soil types in 11 soil series (figure 3 and table I).† Chemical and physical properties of the soils are important ecological limiting factors and determine, in part, the runoff, leaching, and degradation rates of agents tested in the areas; therefore, these characteristics of each soil series are tabulated in tables II and III. The following definitions⁴ are used in these tables and in figure 3:

- 1. Available moisture capacity (inches/inch)—The capacity of a soil to hold water that will not drain away by gravity and that can be used by plants.
- 2. Erosion—The wearing away or removal of soil or geologic material from the land surface, usually by moving water or by wind.
- 3. Erosion class—A measure of extent of erosion (single digit designation on the soil map):
 - a. Class 1 (slight)—Less than 25% of original surface soil removed.
- b. Class 2 (moderate)-Approximately 25% to 75% of original surface soil removed.
- c. Class 3 (severe)—More than 75% of original surface soil and usually part of the subsoil removed.
- 4. Frost action—The heaving of the soil upon freezing; severity depends on drainage and capacity of soil to hold water (poorly drained soils that hold large amount, of water are subject to most severe action).

^{*}Telvar Monuron Weed Killer, E. I. du Pont de Nemours and Company, inc., Wilmington, Delaware.

^{**}The hardened or cemented subsoil layer called "hardpan" also is not a feature of Carroll Island or Grace's Quarters soils.

[†]Soil mapping was performed by the Soil Conservation Service, US Department of Agriculture, in cooperation with the Baltimore County Soil Conservation District. Aerial photographs (Carroll Island, AJO-3DD-299, and Grace's Quarters, AJO-3DD-227) were taken 22 May 1964.

⁴Soil Survey Interpretations for Baltimore County, Maryland. US Department of Agriculture, Soil Conservation Service, Cockeysville, Maryland. April 1966.

Figure 2. Topography of Carroll Island

LOWER ISLAND POINT

The contour interval is 5 feet.

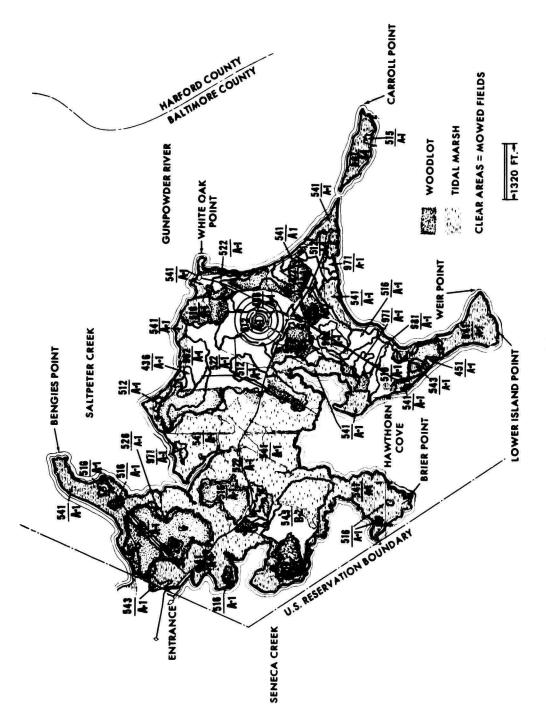


Figure 3. Soil Map of Carroll Island

Three-digit number within each soil area indicates soil type (see table I for index); letter symbol represents the slope of the land within the soil area (see text for definition); single digit number is the erosion class (see text).

Table I. Key to Soil Types Shown in Figure 3 (Three-Digit Numbers on Map)

Soil No. on map	Soil type
432	Sassafras loam
436	Sassafras sandy loam
460	Keyport fine sandy loam
476	Elkton sandy loam
482	Silty and clayey land
512	Woodstown loam
516	Woodstown sandy loam
522	Fallsington loam
526	Fallsington sandy loam
541	Tidal marsh
543	Made land
952	Matapeake loam
962	Mattapex loam
971	Othello silt loam
981	Barclay silt loam

Table II. Some Physical Properties^a of Carroll Island Soils^b

Soil series-No. on fig. 3	Depth to high water table	Overall permeability	Moisture capacity to 30 in.	Probable severity of frost action
	ft		,	
Sassafras-432, 436	5+	Moderate	High	Moderate
Keyport-460	1.5-2	Slow	High	Severe
Elkton-476	0	Slow	High	Severe
Silty & clayey land-482	5+	Slow	High	Moderate to severe
Woodstown-512, 516	2 .	Moderate	High	Severe
Fallsington-522, 526	0	Moderate	High	Severe
Tidal marsh-541	Tidal flooding	_	_	_
Made land-543	_	_		_
Matapeake-952	5+	Moderately slow	High	Moderate
Mattapex-962	2	Moderately slow	High	Severe
Othello-971	0	Moderately slow	High	Severe
Barclay-981	1	Moderately slow	High	Severe

²Terms defined in text.

bSoil Survey Interpretations for Baltimore County, Maryland. US Department of Agriculture, Soil Conservation Service, Cockeysville, Maryland. April 1966.

Table III. Some Physical and Chemical Properties^a of Carroll Island Soils According to Depth

Soil series—No. on fig. 3	Depth from surface	Textural soil group ^b	Permeability	Available moisture capacity	pH (unlimed)	Optimum moisture	Maximum dry density	Shrink-swell potential
	in.	Annual Company of the Company of the Company	in./hr	in./in.		%	lb/cu ft	
Sassafra3-	0-12	SM/ML	2.0-6.3	0.12-0.24	4.0-5.0	_	_	Low
432, 436	12-36	SM/SC/ML/CL	0.63-2.C	0.18-0.24	4.0-5.0	7-18	111-125	Low
	36-60	SP-SM/SM	2.0-6.3	0.08	4.0-5.0	9-15	101-125	Low
Keyport-460	0-8	ML/ML-CL	0.2-2.0	0.18-0.27	4.0-5.0	_	-	Low
	8-40	CL/CII/MH-CH	-0.2	0.18-0.27	4.0-5.0	14-24	101-110	Moderate
···	40-72	SM/SC/ML/CL	0.2-2.0	0.18-0.24	4.0-5.0	10-24	95-125	Low-moderate
Elkton-476	0-10	ML/CL	0.2-2.0	0.18-0.27	4.0-5.0	-	-	Low-moderate
	10-40	CL	-0.2	0.18-0.24	4.0-5.0	16-24	101-110	Moderate
	40-60	SC/ML/CL	0.2-0.63	0.12-0.24	4.0-5.0	10-20	101-125	Low-moderate
Silty, clayey	0-20	ML	0.2-2.0	0.18-0.24	4.0-5.0	12-20	101-110	Low
Land-482	20-96	СН	-0.2	0.18-0.24	4.0-5.0	18-24	91-110	Moderate
Wouldown-	0-11	SM/ML	0.63-2.0	0.12-0.18	4.0-5.0	_	_	Low
512, 516	11-36	SC/SM-SC/CL	0.63-2.0	0.18-0.24	4.0-5.0	7-18	111-125	Low
	36-60	SP-SM/SM-SC	2.0-6.3	0.06-0.10	4.0-5.0	9-15	101-120	Low
Fallsington-	0-10	SM/ML/SM-SC	2.0-6.3	0.12-0.18	4.0-5.0	_	-	Low
522, 526	10-30	SM/SC/ML	0.63-2.0	0.18-0.24	4.0-5.0	10-14	111-125	Low
	30-50	SM/SC/SP	0.63-6.3	0.06-0.10	4.0-5.0	10-14	101-125	Low
Tidal marsh— 541	_	Variable	_	_	5.0-8.0	-	-	-
Made land	-	_	-	_	_	-	-	_
Matapeake-	0-16	ML/ML-CL	0.63-2.0	0.18-0.27	4.5-5.0	_		Low
952	16-34	CL/ML-CL	0.20-0.63	0.18-0.24	4.5-5.0	12-18	101-120	Low-moderate
	34-58	SM/SP-SM	0.63-6.3	0.10-0.18	4.5-5.0	10-15	111-125	Low
	58-62	SP/SP-SM	6.3+	-0.06	4.0-5.0	8-12	91-110	Low
Mattapex-	0-12	ML/ML-CL	0.20-2.0	0.18-0.27	4.5-5.5	_	-	Low
962	12-40	CL/ML-CL	0.20-0.63	0.18-0.24	4.5-5.5	12-18	101-120	Low-moderate
	40-60	SP-SM/SM	0.63-6.2	0.06-0.18	4.0-5.0	10-15	111-125	Low
Othello-	-0-8	ML/ML-CL	0.20-2.0	0.18-0.27	4.0-5.0	_	_	Low
971	8-32	CL/ML-CL	0.20-0.63	0.18-0.24		12-18	111-120	Low-moderate
	32-50	SM	0.63-6.3	0.06-0.12	4.0-5.0	10-14	111-125	Low
Barclay-	0-28	ML/CL/ML-CL	0.20-0.63	0.18-0.27	4.0-5.0	14-24	101-110	Low
981	28-38	ML-CL/CL	0.20-0.63	0.18-0.24	4.0-5.0	10-18	111-120	Low
	38-76	SM,SC	0.63-6.3	0.08-0.18	4.0-5.0	10-14	101-120	Low

^aTerms defined in text.

^bSee text and figure 4.

- 5. Loam-Soil having a relatively equal mixture of sand, silt, and clay.
- 6. Made land—Soils that have been so disturbed by man that normal soil profiles no longer exist (impossible to classify).
- 7. Maximum dry density—The weight in pounds per cubic foot of soil material when compacted by standard methods at optimum moisture content.
- 8. Optimum moisture—The soil moisture content (in percent) at which the greatest compaction can be obtained.
- 9. Permeability—The rate in inches per hour at which water moves downward through saturated soil:
 - a. Rapid-More than 6.3 inches per hour.
 - b. Moderately rapid-Between 2.0 and 6.3 inches per hour.
 - c. Moderate-Between 0.63 and 2.0 inches per hour.
 - d. Moderately slow-Between 0.20 and 0.63 inches per hour.
 - e. Slow-Less than 0.20 inches per hour.
 - 10. pH-A measure of acidity or alkalinity of a soil:
 - a. Below 4.5-Extremely acid.
 - b. 4.5 to 5.0-Very strongly acid.
 - c. 5.1 to 5.5-Strongly acid.
- d. Additional acidic, neutral, or alkaline soils (except for tidal marsh) are not found on Carroll Island.
- 11. Shrink-swell potential—The potential change in volume when a soil is wet versus when it is thoroughly dry.
- 12. Slope—The difference in elevation in percent between two points 100 feet apart within a soil area delineated on the soil map (letter symbol):
 - a. A = 0% to 2%.
 - b. B = 2% to 5%.
 - c. C = 5% to 10%.
 - d. D = 10% to 15%.
 - e. E = 15% to 30%.
 - f. F = 30% or more.

- 13. Soil series—A group of soils similar in all internal characteristics except the texture of the surface soil layer or layers.
- 14. Soil type—A member of a soil series with a specific and relatively uniform texture in the surface soil layer or layers.
- 15. Texture—The relative proportions of sand, silt, and clay particles in the soil; a coarse-textured soil is high in sand, and a fine-textured soil contains a large proportion of clay (see figure 4 for percentages).
 - 16. Water table—The upper limit of soil that is completely saturated with water.

The vegetation of Carroll Isla...J is shown in figure 5. Several of the woodlots were surveyed in 1962 by a forester assigned to Facilities Directorate, Edgewood Arsenal. The dominant tree species are shown in table IV. A comprehensive ecological study of Carroll Island flora is in progress; observations at sampling stations will include species frequency, abundance, and cover and assessments of differences possibly associated with chemical agent contamination.

The estuary surrounding Carroll Island is presently the subject of ecological study. Results of investigations and future research plans are presented in chapters 6 through 10.

Table IV. Dominant Tree Species on Carroll Island

Common name	Generic name
American holly	Ilex opaca
Black cherry	Prunus serotina
Hickory	Carya sp.
Loblolly pine	Pinus taeda
Locust	Robinia pseudacacia
Pin oak	Quercus palustris
Red maple	Acer rubrum
Southern red oak	Quercus falcata
Sumac	Rhus sp.
Sweet gum	Liquidamber styraciflua
Virginia pine	Pinus virginiana
White oak	Quercus alba
Willow	Salix sp.
Willow oak	Quercus phellos
Yellow poplar	Liriodendron tulipifera

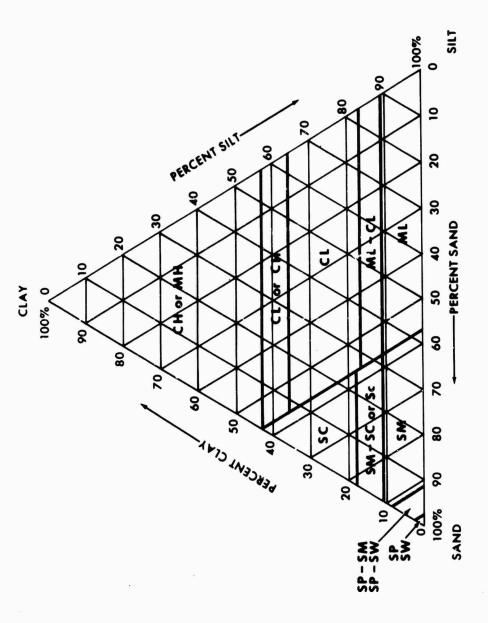


Figure 4. Textural Soil Groups

The composition (silt, clay, and sand) of Carroll Island soils at various depths (texture indicated in column 3 of table III) can be determined. To use the diagram, horizontal lines are projected and read on the "Fercent Clay" side; "Percent Silt" lines are parallel to the clay side of the triangle; and "Percent Sand" lines are parallel to the silt side. For example, a soil texture designated as SC would contain 18% to 42% caly, 0% to 25% silt, and 57% to 82% sand.

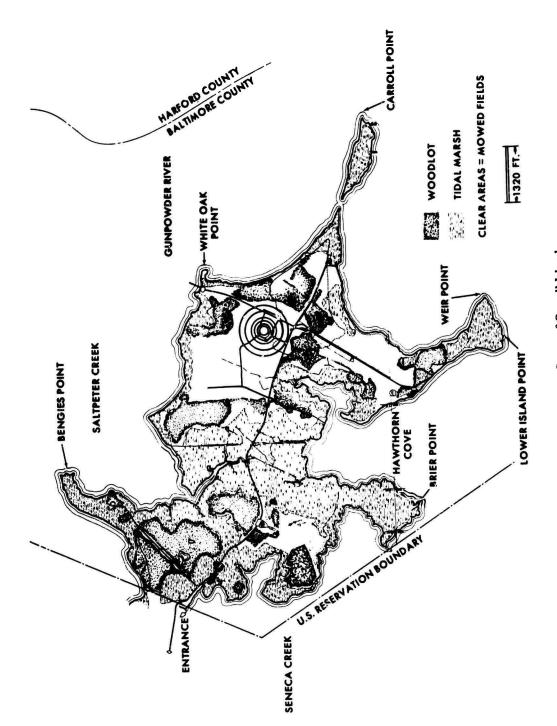


Figure 5. Vegetative Cover of Carroll Island

C. CLIMATE.5-7

Edgewood Arsenal, located near the northern end of the Chesapeake Bay, lies between areas of extreme winter cold to the north and the warmer and more humid summer weather to the south. The climate is warm, temperate, and rainy without a dry season (low temperatures of winter are the major limiting factor for plants rather than drought). Because the area is sheltered by the Appalachian Mountains to the west and because of its close proximity to the Atlantic Ocean to the east, winters are not as cold as those in other locations at the same latitude farther inland.

Edgewood Arsenal lies near the usual path of low pressure systems that move through the area from the west, southwest, and south; these systems contribute to frequent changes in the weather and are responsible for much of the annual precipitation.

During summer months the area is influenced by a large semipermanent high pressure system centered over the Atlantic Ocean near 30° north latitude in the vicinity of Bermuda. The associated flow of warm, moist air from the south contributes to high temperatures and humidity and provides moisture for frequent afternoon and evening thunderstorms. In addition, the Chesapeake Bay causes humidity to be higher at all times of the year than it would otherwise be.

Precipitation is rather uniformly distributed throughout the year, with the heaviest intensities usually in summer and early fall.

January is the coldest month, and July is the warmest. The highest average wind speeds occur during winter and spring, and winds of hurricane force are rare. Snowfalls occur on an average of 25 days each year, with amounts in excess of 1 inch occurring 6 days each year. The heaviest snowfalls are in January, but large accumulations may occur as late as March.

Figure 6 shows mean daily minimum and maximum temperatures for each month and record extreme temperatures during the period May 1959 to June 1968 at Edgewood Arsenal. Table V summarizes other ecologically important climatic factors during the same period.

D. TESTING OF CHEMICAL AGENTS.

1. Carroll Island Test Facilities.8

The Carroll Island Agent Testing Complex is Edgewood Arsenal's principal outdoor test site. The locations of components and support elements of the testing complex are indicated in figure 7.

Four independent test areas—Agent Test Grid 1, Agent Test Grid 2, Aerial Spray Grid, and the Controlled-Velocity Test Chamber (Wind Tunnel)—possess the necessary instrumentation and sampling equipment to permit the simultaneous performance of separate tests.

⁵Harlin, B. W. Final Report. Technical Report ECOM 6039. The Edgewood Arsenal Climate Calendar. Environmental Science Services Administration, US Army Electronics Command, Meteorological Support Activity, Fort Huachuca, Arizona. April 1969. UNCLASSIFIED Report.

⁶Englebrecht, H. H. Climates of the States. Maryland. Climatography of the United States No. 60-18. Weather Bureau, US Department of Commerce, Washington, D.C. December 1959.

⁷US Army Meteorological Team Data, Edgewood Arsenal, Maryland. Monthly Reports, July 1968 to October 1969, inclusive. Meteorological Support Activity, US Army Electronics Command, Fort Huachuca, Arizona. 16 documents. UNCLASSIFIED Reports.

⁸EA Pamphlet 70-2. Research and Development. Carroll Island Agent Testing Complex. 26 January 1970. UN-CLASSIFIED Report.

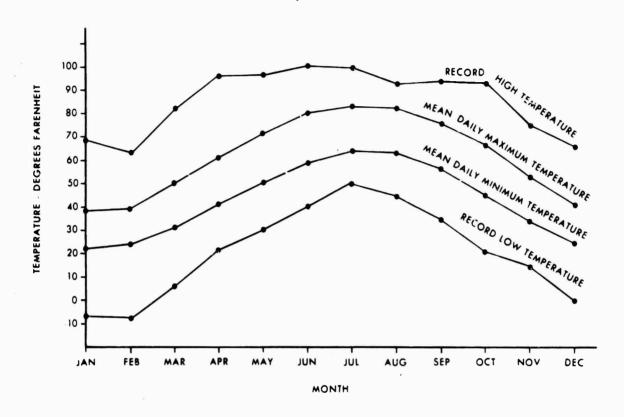


Figure 6. Temperature Means and Extremes at Edgewood Arsenal, Maryland, Compiled From Records
From May 1959 to June 1968

The extremes represent the highest and lowest temperatures for each month during the 9-year period. Temperature means are monthly averages of daily maximum and minimum temperatures for the 9-year period.

Table V. Monthly Summaries of Average Climatological Data for Edgewood Arsenal, Maryland, for a 9-Year Period^a

Daily extreme lapsy ^d		-2.7	- 3.1	-3.6	-3.8	-4.1	-4.2	-4.3	-4.7	4.4	-3.3	-3.1	-2.8
Daily extreme inversion ^d	$_{\bullet}^{F}$	+9.1	+10.9	+8.5	+10.2	+8.5	45.9	1 67	+4.5	+8.2	+8.1	6.6+	+9.3
Soil/snow surface temp ^c		23.041.2	24.3-44.7	30.9-59.4	38.0-71.8	50.4-94.4	60.1-108.3	65.0-113.4	61.8-107.2	54.2-95.1	41.6-75.8	33.4-58.8	26.i 43.2
Station pressure	qш	1018.5	1016.9	1016.1	1015.5	1015.7	1014.9	1014.9	1015.5	1017.0	1017.6	1018.5	1019.1
Dewpoint	J,	22	22	30	38	20	61	2	2	58	94	35	26
Daily solar radiation ^c	Langleys/day	173	244	342	390	481	537	527	451	375	283	. 193	149
Monthly precip ^b	in.	2.33	2.74	3.27	3.34	3.14	3.00	4.27	4.19	3.44	2.08	2.59	2.92
Prevailing wind direction		WNW	WNW	NA	S	MSS	SSW	MSS	S	NNE	N.SS	NW	NNN
Wind speed	ųđш	4	4	\$	5	4	8	8	3	٣	8	4	4
Rel	%	74	71	20	69	71	75	75	77	77	75	72	92
Month		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	oct	Nov	Dec

^aCompiled from records from May 1959 to June 1968.

^bAll precipitation reduced to water equivalent.

^cSoil/ snow surface temperatures are ranges (average daily minimum—average daily maximum).

^dTemperature gradients (inversion, lapse) measured between 0.5 and 4.0 meters.

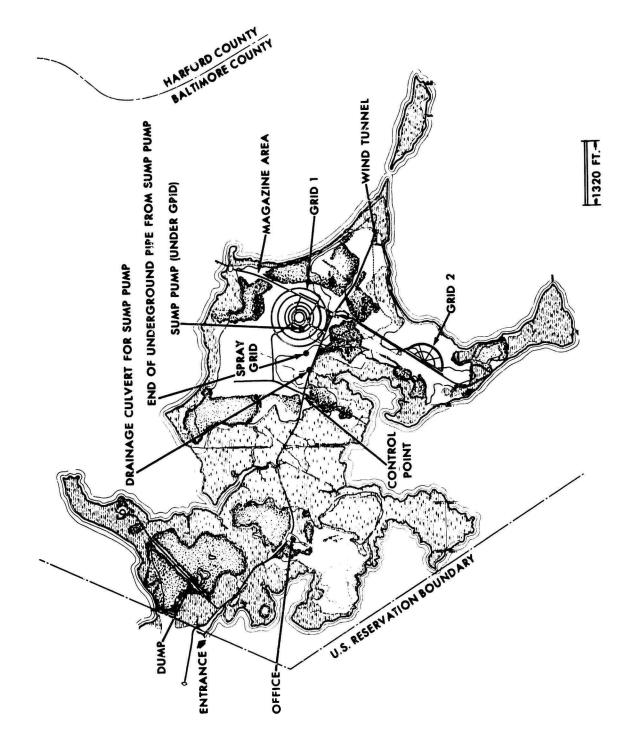


Figure 7. Locations of Carroll Island Test Sites and Support Facilities

Agent Test Grid 1 is located on a 50-acre mowed site. It is used primarily for establishing dissemination efficiency and area distribution estimates of agent aerosols and vapors, for comparing munition performances under various meteorological conditions, and for acquiring quantitative information on field behavior of chemical agents. The grid includes an integrated complex of underground vacuum systems, electric power and control systems, chemical sampling stations, a micrometeorological network, and associated instrumentation.

Samplers for measuring concentration and distribution of aerosols and vapors are located along the circumferences of eight circles at radii of 20, 30, 40, 50, 75, 100, 150, and 200 yards from grid center. Each sampler has its own vacuum and power source. Motion picture cameras are placed on the 150-yard circle at 45°, 135°, 225°, and 315° from true north to record mover tent of the agent cloud. High-speed cameras (2000 frames per second) are located behind barricades near grid center to photograph bursting characteristics of munitions. All operations are controlled from, and meteorological conditions are monitored and recorded at, a central control point.

The meteorological tower is located in the northwest quadrant of the 75-yard circle. Sensors are mounted at eight heights (0.5, 1, 2, 4, 8, 16, 32, and 64 meters) on the 200-foot tower; they measure temperatures and wind speeds and show wind direction.

A 60-foot drop tower is used to determine whether test items can withstand shocks induced by loading and unloading.

Wind profile stations are located on the 20-, 30-, and 100-yard circles (seven stations per circle); they measure wind speeds at heights of 0.5, 1, 2, 4, and 8 meters and monitor wind direction at 2 and 8 meters. Temperature profile stations are located at 0°, 90°, and 180° from true north on the 50-yard circle; thermosensitive elements are placed at heights of 0.5, 1, 2, 4, and 8 meters.

An inner sampling system on Grid 1 (figure 8) covers the surface area between grid center and the 20-yard circle with 172 individual ground-level samplers. A drainage system and sump pump prevent flooding of the underground elements of this system.

A vertical sampling system consists of 64 masts that can be equally spaced on the 20- or 30-yard circle; each mast is equipped to measure the density of agent clouds at heights of 0.5, 1, 2, 3, 4, 5, and 8 meters. A horizontal sampling system consists of 512 sampling positions equally spaced along the circumferences of the eight sampling circles.

A 40-foot high A-frame, located at grid center, is used to suspend munitions for test firing at various heights above the ground.

Agent Test Grid 2 is used to assess dissemination efficiency and the behavioral characteristics of small amounts of agent. Capabilities of the grid include measurement of airborne aerosols and vapors and measurement of contamination. This general area is also used for vegetation-contamination studies and terrain denial investigations.

The Aerial Spray Grid is adjacent to, and northwest of, Grid 1. The area is used for testing airborne spray devices and munitions using agent simulants or riot control agents and for terrain denial studies.

The Controlled-Velocity Test Chamber or Wind Tunnel is used to study behavior of aerosols, to determine efficiency of thermogenerating devices, to calibrate new sampling equipment, and to study the vaporization efficiency of agents. The tunnel has also been used as a static



Figure 8. A Portion of the Ground Sampling System, Agent Test Grid 1

The tall masts in the background are part of the vertical sampling system at the 20-yard circle.

diffusion chamber, for protective mask studies, and for controlled agent exposures of animals and man. Wind speeds can be varied from 2 to 20 miles per hour. The tunnel is divided into three sections:

- a. The mixing section where the munition is exploded and the products are mixed with the incoming air.
 - b. The test section where samples of air are taken by vacuum sampling devices.
 - c. The exhaust section, consisting of blower and exhaust stack.

Support and miscellaneous facilities on Carroll Island include an office, a burning pit for agents and munitions (located at the northwest corner of the Spray Grid), a dump for obsolete equipment and used containers, a magazine area for long-term storage of toxics and pyrotechnic mixes, a personnel decontamination station, and assorted observation towers, generators, and equipment storage buildings.

2. Types of Agents Field-Tested.

A chemical agent is a solid, liquid, or gas that, through its chemical properties, produces lethal or damaging effects on man, animals, plants, or materiel or produces a screening or signaling smoke. Since field testing began on Carroll Island in 1947, the following types of chemical agents, classified according to physiological actions, have been field-tested; anticholinesterases, incapacitants, riot control agents (emetic agents and tear agents), blister agents, choking agents, and blood agents. In addition, chlorine, simulant and training agents, screening and signaling smokes, and incendiaries have been field-tested on Carroll Island (no blister agents except distilled mustard, no chlorine, no choking agents, and no blood agents have been disseminated on Carroll Island during the past 5 fiscal years).

Information about agents and simulant agents, including physiological actions, chemical formulas, and various physical and chemical properties, is detailed in other publications.^{9,10} Only anticholinesterases, incapacitants, and riot control agents have been disseminated in significant quantities during the past 5 fiscal years.

3. Summary of Safety Procedures.

The Carroll Island/Grace's Quarters field-testing complex is manned by personnel of Field Evaluation Division, Technical Support Directorate. Requests for tests are initiated by Research Laboratories, Weapons Development and Engineering Laboratories, or Defense Development and Engineering Laboratories. Occasional trials are also conducted by Technical Support Directorate to calibrate their test equipment. Each request is reviewed to validate the need for the field trial.

A standard operating procedure (SOP) delineates general responsibilities, procedures, controls, and safety requirements for conducting open-air tests of lethal, incapacitating, and riot control agents at Edgewood Arsenal.¹¹ This general SOP must be used in conjunction with

⁹Military Chemistry and Chemical Agents. Department of the Army Technical Manual 3-215 (December 1963) with Change No. 1 (16 March 1965) and Change No. 2 (15 June 1967). Department of the Army, Washington, D.C. 1-51.

¹⁰TB MED 282. Anticholinesterase Intoxication: Pathophysiology, Signs and Symptoms and Management. Department of the Army, Washington, D.C. 21 January 1970. UNCLASSIFIED Report.

¹¹TSDSOP No. 4-2-69-17. Open Air Testing of Lethal, Incapacitating and Riot Control Agents at Edgewood Arsenal, UNCLASSIFIED Report.

additional SOP's that have been developed for conducting each type of test. These additional SOP's include specific precautions to be observed with each agent and munition, protective clothing to be worn by those conducting the test, exact micrometeorological requirements, and special safety precautions.

By Edgewood Arsenal regulations, ^{12,13} all open-air lethal and incapacitating tests are conducted on the Carroll Island or Grace's Quarters sites, with very limited exceptions.* Riot control agent tests may be conducted on parts of Gunpowder Neck. (The following testing restraints were employed before lethal agent tests were discontinued in July 1969, and will be applied to the same tests if they are resumed.)

Whether a specific test is conducted depends on certain prevailing meteorological conditions at the test site. Wind direction, wind speed, temperature gradient (inversion, neutrality, or lapse), and the total quantity of agent are considered in formulating testing restraints. Safety distance tables 11 for all agents dispersed in open-air tests are based on human "no effect" maximum dosage estimates furnished by Medical Research Laboratory. Research Laboratories. Downwind safety distances are increased by light winds, by an atmospheric inversion, or by larger amounts of agent to be disseminated.

Critical test zones, depending on stable wind direction, have been established, and specific safety limitations have been delineated for each zone. A stable wind direction exists if the direction, measured at the 2-meter high sampler, has not diverged from the recorded mean by more than $\pm 15^{\circ}$ for a 5-minute period prior to test. The test engineer in charge insures that sufficiently stable meteorological conditions prevail at the test site to permit functioning of the dissemination device. Additional testing restraints are as follows:

- a. Disseminating lethal or incapacitating agents from aircraft or from an elevated line source above 75 feet is prohibited. Aerial dissemination of riot control agents is permitted, subject to all applicable provisions of safety SOP's.
- b. Tesis of lethal agent devices are conducted statically, or devices are projected at limited ranges from test fixtures (generally less than 500 meters); this type of projection is contrasted to firing at long ranges from gun tubes.
- c. Testing is normally limited to single rounds or submunitions unless grouping is required to produce enough agent to provide a measurable effect.
- d. The maximum amount of agent disseminated is governed by the downwind safety distance tables mentioned previously. 11
- e. Most lethal and incapacitating agent tests are conducted at ground level or at a height of 10 feet or less. Projection or static detonation from 10 feet to 75 feet above ground level is permitted provided that, between 59 and 75 feet, at least 75% of the total quantity of agent is disseminated as particles of at least 100 microns in mass median diameter. This insures rapid fallout of droplets, confining the agent to a localized area within the sampling grid.
- f. Tests are conducted so that prevailing meteorological conditions will insure that an agent cloud remains within the boundary of the military reservation until it is sufficiently diluted so as not to present a hazard to, nor have any effect upon, the general human population of the surrounding area.

^{*}Aberdeen Proving Ground, for example, occasionally has granted special permission for the use of 1 field bunkers on Gunpowder Neck for tests of shaped-charge warheads containing agent.

¹²EA Regulation 10-1. Organization and Management Manual. 7 July 1965. UNCLASSIFIED Report.

¹³EA Regulation 385-3. Safety. Range Areas. 3 April 1968. UNCLASSIFIED Report.

- g. Water areas within a downwind danger zone are protected by Army patrol craft to prevent intrusion by civilian beats.
- h. The danger zone anticipated from any test at Carroll Island or Grace's Quarters involving lethal or incapacitating agents is always projected away from the western boundary of the military reservation. The maximum safety limit is 3500 meters.
- i. When successive trials are conducted with agents that have cumulative toxic effects, the safety distance is calculated on the basis of total quantity of agent disseminated per day. The maximum amount of agent dispersed is recalculated when a definite wind shift relocates the danger zone into another acceptable testing zone.

When a test is concluded, possibly contaminated areas are indicated by barriers, signs, or tape; these markings are maintained until evaporation, weathering, or decontamination procedures have removed the agent. Analyses of samples taken from the area periodically determine when contamination has decreased to safe levels. If necessary to minimize possible secondary vapor effects, the test engineer may conduct decontamination procedures on grossly contaminated portions of the test area. Personnel and patrols are used as necessary to maintain security and to prevent human access to the danger area.

Peripheral sampling stations using mechanical air samplers and biological monitors (sentry animals) and water, soil, and vegetation sampling stations have been established on Carroll Island. Placement of samplers and monitors and frequency of obtaining samples depend on type and amount of agent being tested, downwind safety distance, dissemination technique, wind direction, and other special considerations such as rainfall following a test, shifts in wind direction, or changes in atmospheric stability.

4. Summary of Agent Tests Performed During Preceding 5 Fiscal Years.

Figures 9, 10, and 11 show total monthly quantities of lethal agents, incapacitants, and riot control agents respectively released on Carroll Island from July 1965 to June 1970. Table VI summarizes miscellaneous tests and procedures occurring in each of the past 5 fiscal years.

Only anticholinesterases have been tested on Grace's Quarters during the past 5 fiscal years; the pounds dispersed are as follows (the first number in parentheses is the number of trials; the second number is test days):

- a. FY66-189.2 (25/23).
- b. FY67-0.15 (7/5).
- c. FY68-0.07(3/3).
- d. FY69-2.4 (5/5).
- e. FY70-1.1 (1/1).

Also, 25 pounds of herbicide* were used on 19 July 1967 around buildings and other structures on Grace's Quarters.

^{*}Telvar^R Monuron Weed Killer, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware.

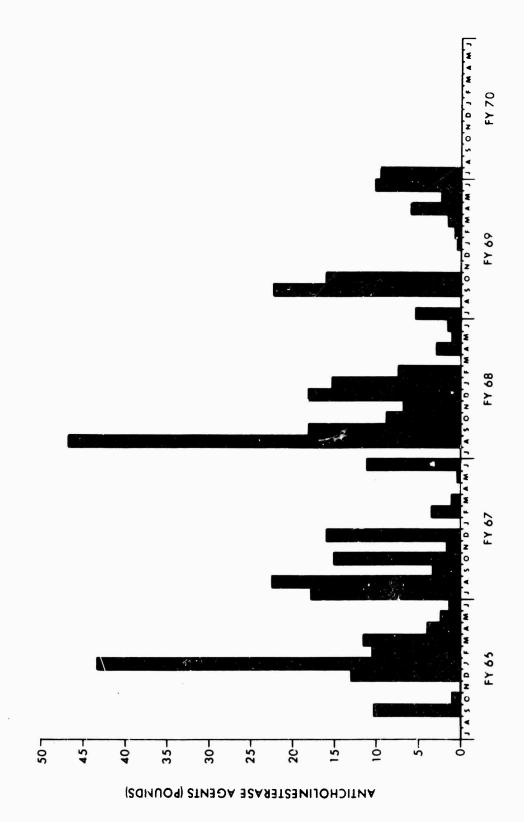


Figure 9. Amounts of Lethal Agents Disseminated in Carroll Island Tests During the Past 5 Fiscal Years

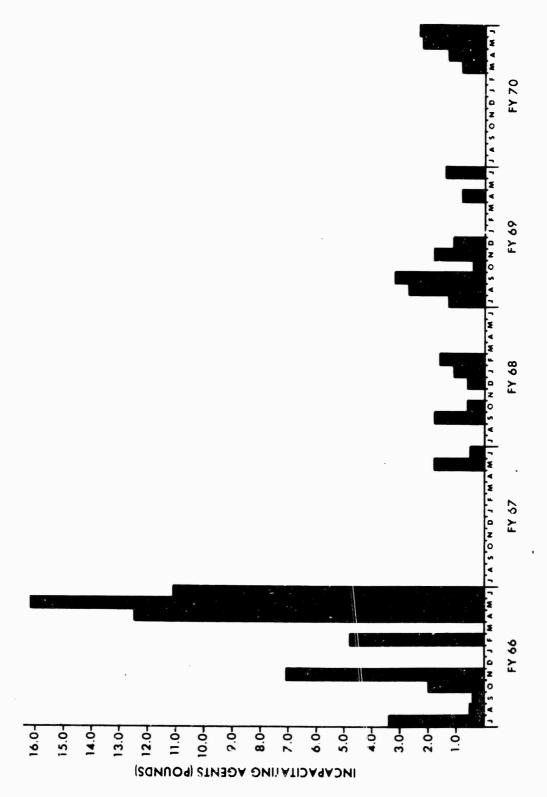


Figure 10. Amounts of Incapacitating Agents Disseminated in Carroll Island Tests During the Past 5 Fiscal Years

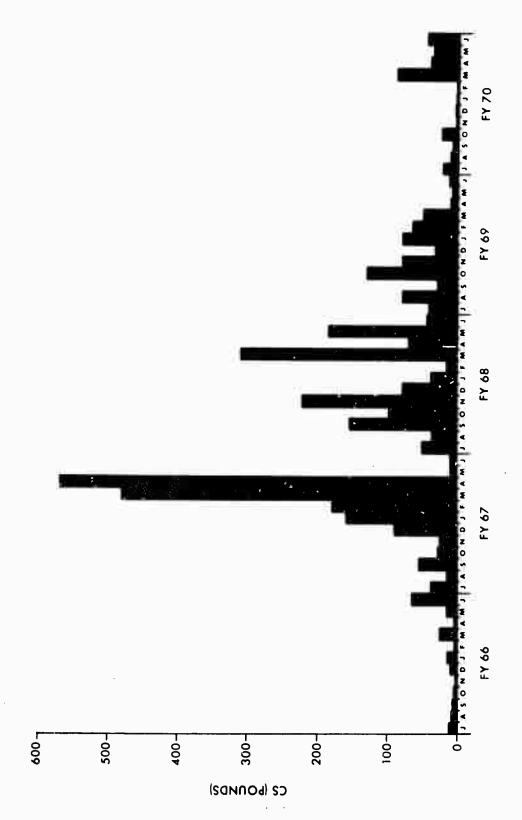


Figure 11. Amounts of CS (Tear Agent) Disseminated in Carroll Island Tests During the Past 5 Fiscal Years

able VI. Miscellaneous Tests and Procedures on Carroll Islanda

Agent or	Pounds dispersed					
compound	FY66	FY67	FY68	FY69	FY70	
CAb	4.8 (4)	0.9(1)	0	0	0	
CNb	4.0(3)	0	0	0	0	
DMb	7.1 (4)	0.5 (2)	0	0	0	
CN/DM ^b	179.6 (39)	1.6(1)	0	0	0	
CS/DMb	133.2 (26)	1.0(2)	0	0	0	
Distilled mustard	0	0	0	7.6 (4)	0	
White phosphorus	0	28.5 (3)	122.0 (4)	3.0(3)	3.0(1)	
FS (screening smoke)	0	0 1	2.2(1)	4.4 (2)	2.2 (1)	
TEA (incendiary)	0	0	0	1.5 (1)	12.0(2)	
Talc (simulant)	0	1603 (2)	0	600.0(1)	3017.5 (29)	
Herbicide ^c	150.0	50.0	0	50.0	50.0	

^aNumber in parentheses = number of test days.

5. Environmental Contamination.

We know that significant contamination of portions of Carroll Island occurs; investigations in our ecology program are designed to measure this terrestrial contamination and to insure that the surrounding estuary is not significantly contaminated.

The persistence of agent and the ecological impact of Carroll Island agent tests are the cumulative result of several factors.

a. Type of Agent Tested.

The agents and simulants tested differ greatly in physical characteristics and toxicities. For example, CS (tear agent) and VX (anticholinesterase), although quite different in mode of action and toxicity, are relatively persistent on soil and vegetation. In contrast, GB (anticholinesterase) volatilizes almost immediately after release.

b. Dissemination Technique.

The method in which the agent is released includes considerations of the munition or device used, the height of dissemination, the particle size of the aerosol, and the quantity of agent used.

The most toxic agents tested were the anticholinesterases, of which VX appears to be the most stable in the environment.² The extent of environmental contamination resulting from disseminating 6.2 pounds of VX contained in a 155nm artillery shell at Test Grid 1 was investigated.*

As the agent cloud passed through the 20-yard circle of samplers, 15% of the original quantity of VX was airborne.

bRiot control agent.

^cTelvar^R Monuron Weed Killer, E. I. du Pont de Nemours and Company, Inc., Wi'mington, Delaware.

^{*} Air temperature was 64°F, wind was NW at 8.6 mph, and temperature gradient was -2.3°F.

Ground deposition of VX within the 20-yard circle accounted for 60% of the total quantity. If 60% was on the ground and 15% passed through the first circle, then apparently 25% was thermally decomposed by the heat of detonation of the munition. Of the 15% that was airborne 20 yards from grid center, 5% was deposited on the ground beyond this point.

Thus, 10% of the original quantity of VX degraded before soil samples were taken, was deposited in undetectably low concentrations, or was of sufficiently small particle size that it escaped the perimeter of Carroll Island.*

c. Testing Site.

Because of the nature of the trials that occur at each of the four test sites, contamination of these areas varies. The quantities of lethal, incapacitating, and riot control agents used in wind tunnel tests, for example, were discharged into the atmosphere through an exhaust stack (a scrubber system, described in chapter 4, is due to be installed in the stack in FY71).

Grids 1 and 2 accumulated similar quantities of the tear agent CS and anticholinesterase agents. The Spray Grid has received the greatest quantities of CS and anticholinesterases during the past 5 years.

The annual contamination of the four Carroll Island test sites for the past 5 fiscal years is shown in tables VII, VIII, IX, and X; numbers of trials and testing days are also indicated.

A dump (figure 7) is a possible minor source of contamination, for the site has been used to dispose of empty agent containers and munitions and obsolete test equipment and sampling devices. Agents and munitions are occasionally destroyed at the burning pit.

d. Meteorological Variables.

Meteorological variables can significantly alter the fate of agents in the environment. Low temperatures, for example, greatly increase the persistence of distilled mustard, and atmospheric inversions impede the dispersal of vapors or fine aerosols.

The estuary surrounding Carrell Island could be contaminated in several ways:

- (1) Droplet fallout from an agent cloud (most fallout occurs in the immediate vicinity of grid center).
- (2) Rainwater runoff from contaminated land areas (drainage from all test areas is generally in the direction of Hawthorne Cove).
- (3) Water that runs into the inner grid ground samplers of Test Grid 1 drains into a sump pump, which, when full, forces possibly contaminated water through an underground pipe (figure 7). This pipe ends underground about 350 yards WSW of grid center. The effluent then presumably percolates to the soil surface where its contained agent degrades or it is washed into a roadside ditch, through a culvert, and into Hawthorne Cove.

A sampling and monitoring program (chapter 7) is designed to explicitly define the extent and persistence of environmental contamination. Additional studies are determining the movement, concentration, toxicity, and fate of agents in the ecosystem.

^{*}Downwind safety distances are based on the assumption that the total quantity of agent contained in each munition escapes from the test area in an agent cloud. These safety estimates are based on human "no effect" levels and do not consider sensitivities of other organisms.

Table VII. Pounds of Agent Disseminated at Agent Test Grid 1
During the Past 5 Fiscal Years

In tables VII through X, the first number in parentheses is the number of trials, and the second is the test days.

Fiscal year	Anti-ChE's	Incapacitants	CS (tear agent)
		lb	
1966	13.3 (7/7)	0	7.0 (4/2)
1967	14.8 (10/6)	0	129.9 (30/14)
1968	41.4 (22/20)	0	47.3 (25/25)
1969	18.5 (3/3)	0	60.2 (50/29)
1970	9.2 (1/1)	0.9 (4/2)	50.3 (155/48)

Table VIII. Pounds of Agent Disseminated at Agent Test Grid 2

During the Past 5 Fiscal Years

Fiscal year	Anti-ChE's	Incapacitants	CS (tear agent)
		lb	
1966	32.8 (6/3)	0	0
1967	6.0 (3/3)	0 .	0 ·
1968	19.7 (7/7)	0	285.8 (106/9)
1969	6.3 (12/4)	0.04 (4/4)	53.2 (134/22)
1970 .	0.01 (1/1)	0	16.7 (23/9)

Table IX. Pounds of Agent Disseminated at Aerial Spray Grid During the Past 5 Fiscal Years

Fiscal year	Anti-ChE's	Incapacitants	CS (tear agent)
		lb	
1966	50.9 (13/6)	2.4 (5/2)	66.9 (226/11)
1967	59.3 (59/16)	2.3 (52/3)	1277.2 (547/48)
1968	56.6 (35/8)	0	696.7 (600/35)
1969	37.4 (35/10)	4.0 (10/2)	286.8 (679/61)
1970	0	0	161.2 (243/26)

Table X. Pounds of Agent Disseminated in Controlled Velocity
Test Chamber During the Past 5 Fiscal Years

Fiscal year	Anti-ChE's	Incapacitants	CS (tear agent)
		lb	
1966	0	55.7 (264/26)	87.6 (811/69)
1967	12.3 (23/11)	0	248.9 (1005/71)
1968	8.0 (8/3)	5.7 (126/11)	267.2 (731/77)
1969	1.9 (157/26)	8.5 (189/16)	213.4 (679/61)
1970	0.2 (2/2)	0	54.3 (515/35)

CHAPTER 6

CHOLINESTERASE MEASUREMENTS

1LT George D. Edwards, Everett A. Haight, and F. Prescott Ward were principal investigators in several studies within this portion of the program.

A. SENTRY ANIMALS.

The use of biological monitors in conjunction with the anticipated resumption of the lethal agent testing program was initiated in response to Bennett Committee recommendation 1.a.

Since the biochemical lesion caused by exposure to G and V agents is inhibition of cholinesterase (ChE), our monitoring program is based primarily on ChE measurements. The method used is a modification of the automated colorimetric procedure described by Ward and Hess. ¹⁴ Cholinesterase in the sample (erythrocytes, plasma, brain, and other tissue homogenates) hydrolyzes the substrate acetylthiocholine (ASCh) to produce acetic acid and thiocholine. A chromogenic indicator of sulfhydryl groups then combines with thiocholine, and the intensity of the resulting yellow color is automatically recorded.

During FY70, baseline data were obtained on three sentry species (sheep, white perch, and starlings) to be used in the Carroll Island monitoring program (no lethal agent field test occurred after 14 July 1969).

1. Sheep. (Investigations conducted by 1LT George D. Edwards.)

Sheep were chosen to be used as sentries for the following reasons:

- a. As herbivores they would be exposed directly to intoxication by ingesting possibly contaminated forage.
- b. The Army had accumulated large amounts of toxicological data on sheep as a result of investigations following the Dugway incident.
 - c. Sheep are easily transported, handled, and confined.
- d. Blood samples are readily obtained, and both erythrocytes and plasma have measurable quantities of ChE that are sensitive to anticholinesterases.
 - e. Sheep are inexpensive, long-lived, and tolerate temperature extremes well.

We defined optimum conditions for measuring ChE in samples of red blood cells (RBC) and plasma from 20 sliep that had been procured for use as sentries.

2. White Perch (Roccus americanus). (Investigations conducted by 1LT George D. Edwards.)

This species was chosen as the primary estuarine indicator species because:

¹⁴Ward, F. P., and Hess, T. L. Automated Cholinesterase Measurements: Canine Erythrocytes and Plasma. Amer. J. Vet. Res., 32 (3): 499-503 (1971).

- a. White perch are commercially valuable.
- b. They are abundant during all seasons in waters surrounding Carroll Island.
- c. They are easily obtained by a number of netting and seining procedures.

Optimum conditions were determined for measuring ChE in white perch brain homogenates. Normal levels (95% confidence interval estimates) are being established considering the following variables (based on data accumulated on 738 white perch brain ChE measurements as of 30 June 1970):

a. Brain Weight.

Small fish have higher brain ChE than large fish.

b. Sex.

There is no significant brain ChE difference in spawning males versus females captured in February and March 1970.

c. Freezing.

A significant decrease in brain ChE (15% to 20%) is produced by freezing the fish or excised brains at -70°F; there is no correlation between duration of freezing and decrease in ChE activity.

d. Autolysis.

In early March 1970, 100 dead white perch were placed in a sack in the Gunpowder River. Ten fish were removed daily, and brain ChE was measured in the decomposing specimens. Levels of brain ChE decreased to 60% to 70% of normal during the first 3 days, then did not decrease further. The initial loss of ChE activity may have been a result of a dilution error caused by brains imbibing water after death.

e. Confinement.

Results of preliminary studies indicate that after 48 hours of confinement in fish exposure pens, white perch have about 10% more brain ChE than freshly caught specimens.

f. Seasonal Periodicity.

These investigations have been hampered because of the seasonal migrations of different age classes of white perch. For example, large spawning individuals (about 350 to 450 grams body weight) are plentiful in February and March, but they migrate from the area in summer. Small fish (50 to 100 grams body weight) are plentiful in the area only in summer, and young-of-year are available in summer and fall. Results of initial investigations, however, indicate that there is an increase in brain ChE levels as water temperature increases during the summer.

Initial studies designed to correlate brain ChE depression with anticholinesterase intoxication and death were performed with malathion. Because of errors in holding and exposure techniques, deaths were variously scattered through all groups (including controls); but brain ChE depression in the limited samples correlated reasonably well with increasing concentrations of the insecticide (figure 12).

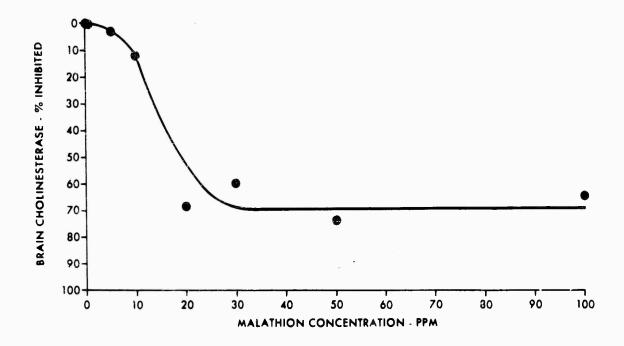


Figure 12. Brain Cholinesterase Inhibition in White Perch Exposed to Malathion (10 Perch Per Dose)

White perch studies are nearly completed, and a report on these studies is in preparation.

3. Starlings (Sturnus vulgaris). (Investigations conducted by Everett A. Haight.)

Because of their abundance, availability during all seasons, and comparatively unrestricted food preferences, starlings were chosen as free-living monitors for brain ChE measurements. They were to be collected quarterly from each of the four Carroll Island test sites and a control area. In FY70 preliminary work to define optimum conditions for measuring ChE was accomplished.

B. SPECIES OF COMMERCIAL AND AESTHETIC VALUE.

1. Blue Crab (Callinectes sapidus). (Investigations conducted by F. Prescott Ward.)

The blue crab has been the subject of intensive investigations in our laboratory because of its tremendous commercial value in the Chesapeake Bay area. Basic ChE studies were designed to develop a way to determine rapidly whether dead crabs were victims of anticholinesterase intoxication.

Three sources of ChE from blue crabs were considered.

a. Hemolymph samples were obtained from the infrabranchial sinus of the fifth periopod¹⁵ with a needle and syringe using EDTA* as an anticoagulant (when heparin or no

¹⁵ Pyle, R., and Cronin, E. The General Anatomy of the Blue Crab. Callinectes sapidus Rathbun. State of Maryland, Board of Natural Resources, Department of Research and Education. Chesapeake Biological Laboratory Publication 87. August 1950.

^{*}Dipotassium ethylenediamine tetra-acetate, Sequester-Sol^R, Cambridge Chemical Products, Inc., Detroit, Michigan.

anticoagulant was used, hemolymph clotted). However, measurable ChE activity of hemolymph was too low to be of practical significance. Also, hemolymph would be difficult to retrieve from a dead crab.

- b. The largest and most accessible nervous structure in the blue crab is the thoracic ganglion.¹⁵ Because we had difficulty in excising this organ from fresh specimens, it too was discarded as a source of enzyme.
- c. Muscle tissue (containing probably all neuromuscular enzyme) provided a concentrated and accessible source of ChE. The fifth periopod was pulled from freshly killed crabs, and a small portion of the remotor muscle (about 100 mg) was snipped off for ChE measurements.

Current investigations are determining normal ChE levels and considering such variables as sex, autolysis, size (age), molt-stage, and seasonal variation. The ChE seasonal variation to date in intermolt adult male blue crabs is shown in figure 13. As water temperature increases, so does activity of the crab and ChE.*

Initial investigations to correlate ChE depression with anticholinesterase exposure and death were performed with malathion. Figure 14 shows crab muscle ChE depression related to a 24-hour malathion exposure; even at the solubility limits of malathion, muscle ChE was only moderately inhibited (2 of 10 crabs died at the highest concentration). These investigations should be completed during next fiscal year.

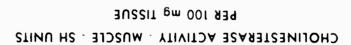
2. Other Species.

Preliminary work has been accomplished on defining optimum conditions for measuring brain ChE in cottontail rabbits (Sylvilagus floridanus), Canada geese (Branta canadensis), mallard ducks (Anas platyrhynchos), whistling swans (Olor columbianus), pumpkinseed sunfish (Lepomis gibbosus), and snapping turtles (Chelydra serpentina).

^{*}The February samples were obtained through the excellent cooperation of Robert Lippson and the crew of the Research Vessel *Orion*, Chesapeake Biological Laboratory, Natural Resources Institute, University of Maryland. Crabs were dredged on 17 February 1970 in the upper Chesapeake Bay near the mouth of the Choptank River.

Figure 13. Seasonal Variation (to Date) of Muscle Cholinesterase of Blur C.abs

Number in parentheses is number of samples.



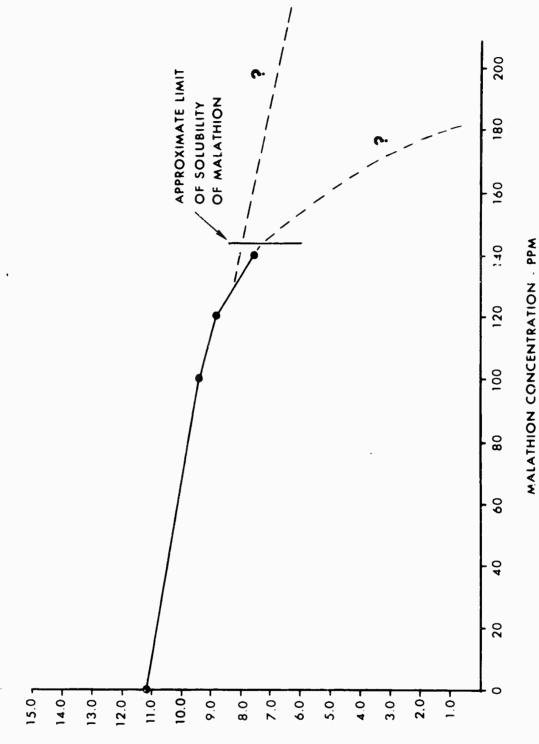


Figure 14. Muscle Cholinesterase Inhibition in Blue Crabs Exposed to Malathion (10 Crabs per Dose)

CHAPTER 7

MONITORING

Principal investigators conducting studies in this portion of the program were 1LT George D. Edwards, Abraham Koblin, Samuel Sass, and Robert T. Howe.

The monitoring program on Carroll Island has been designed to implement Bennett Committee recommendation 1.a., which encourages the more frequent use of mechanical and biological monitors in conjunction with lethal agent tests. Baseline data have been accumulated during FY70; placement of sentry species and initiation of sampling schedules for soil, vegetation, water, and air discussed in this chapter depend on the resumption of lethal agent testing.

A. LOCATIONS OF SAMPLING STATIONS.

Dissemination of lethal and incapacitating agents is prohibited when prevailing winds move an agent cloud toward the Carroll Island boundaries northwest, west, or southwest of Agent Test Grid 1. Small quantities of riot control agents may be dispersed in this zone if meteorological conditions insure that the agent will be contained within the boundaries of the military reservation. Riot control agents, however, are not tested at Grid 1 when winds are directly from the east (buildings and other obstructions west of the grid would interfere with normal movement of the agent cloud).

Thus, all this area should be free of lethal or incapacitating agent contamination, and a 20°-arc directly west of Grid 1 should be free of all agent contamination.

Before lethal agent tests were suspended on Carro'! Island, the area southeast of Agent Test Grid 1 received the heaviest deposition of anticholinester ses, for nearly all tests at Grid 1 and the Spray Grid were conducted with the wind blowing in this direction.

Sites for monitoring stations were chosen so that zones of heavy, intermediate, and no lethal agent contamination could be compared. Locations for housing sentry animals and for obtaining environmental samples are shown in figure 15.

Sentry sheep will be confined between tests in a large holding pen behind the Carroll Island office. Five sheep exposure pens, each 50 by 50 feet, have been constructed. Four pens encircle the Spray Grid-Test Grid 1 mowed site, and a fifth pen is located directly east of Test Grid 2. Five of the land sampling stations for obtaining soil and vegetation are within the five sheep experimental pens. Additional land stations are located near the holding pen (no contamination), and between the 30- and 40-yard circles of Grid 1 (heavy contamination).

Six sites for anchoring floating fish cages surround Carroll Island. Each cage, 3 by 3 by 3 feet, is constructed of ¼-inch mesh nylon netting. During initial investigations with the cages, we discovered that great blue herons would often perch on the floating rims and prey on the confined fish. Therefore, lids of ¼-inch mesh hardware cloth have been added to each cage. Experiments have been conducted to measure baseline brain ChE levels in white perch and variations in this enzyme caused by confinement in the pens (chapter 6). Additional investigations are being conducted on pumpkinseed sunfish (to be used as an alternate monitor species).

Water sampling stations are located at the six fish exposure sites. In addition, water samples will be obtained periodically from the sump pump under Grid 1 and from the roadside ditch WSW of grid center (this ditch collects runoff from the grid and piped effluent from the sump pump).

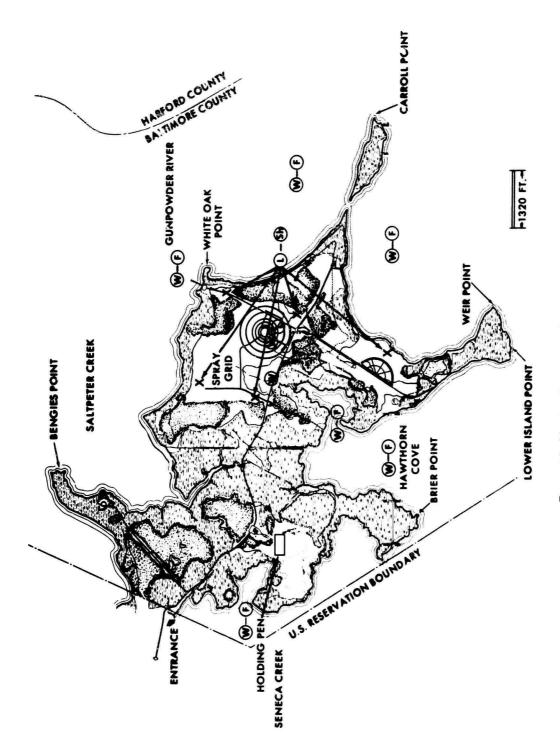


Figure 15. Monitor Stations on Carroll Island

Windicates water sampling, F designates locations for fish exposure cages, L refers to land sampling stations for obtaining soil and vegetation samples, and Sh means sheep exposure pen.

Another monitoring site is located on Gunpowder Neck, due east of Carroll Island Agent Test Grid 1. This site was chosen because it is the closest downwind land mass from the grid. Sentry sheep will be housed in a pen 50 by 250 feet at a soil and vegetation sampling site, and a fish exposure cage will be anchored offshore at a water sampling station. Air samplers will be placed at each land and water station (except the sump pump).

The exact locations of all monitoring sites are tabulated in the appendix. This monitoring program augments routine environmental sampling using mechanical samplers that already existed on Carroll Island and incorporates sampling procedures that were initiated in response to Bennett Committee recommendations.

B. SAMPLING SCHEDULES.

On the day preceding each lethal agent test, 10 white perch (or pumpkinseed sunfish if sufficient white perch are not available) will be placed in each of the fish exposure pens, and brain ChE will be measured in 15 additional control fish. One gallon of surface water will be obtained from each water station, including the sump pump and drainage ditch when available. Lethal agent residues will be measured in each sample, and organisms composing a laboratory aquatic food web (chapter 8) will be exposed to a portion of each water sample.

Several cores of soil, 3 inches in diameter and 6 inches deep, will be obtained from each land station. Composites of vegetation, roots, and subsurface soil will be prepared from each station's samples, and lethal agent residues will be measured in the subsamples.

Blood samples for RBC and plasma ChE measurements will be obtained from each sentry sheep. These animals will remain in the holding pen until immediately before the test; the test engineer will then decide, based on meteorological conditions, which exposure pen or pens are likely to become contaminated. Sheep assigned to pens in safe areas will be placed in these pens prior to the test. Air samplers located at all land and water stations will be turned on just before the test.

As soon as possible after the lethal agent trial, soil and water samples will be retrieved from all stations. Any dead fish will be removed from the cages for brain ChE measurements. One-half of the fish remaining in each cage will be removed 24 hours after the test and one-half at 48 hours. Soil and water samples, sheep blood samples, and air samples will be taken every 24 hours as long as evidence of a toxic agent persists.

When the test engineer decides (based on analytical results and knowledge of agent degradation) that a contaminated area is safe for normal human activity, assigned sheep will be moved to pens in this area. These animals will be observed for toxic signs, and blood samples will be taken every 24 hours. If any animal shows signs of illness or an individual's RBC ChE falls below 50% of normal, the group will be removed. Deep core samples and additional air samples will then be taken from the area to determine the source of agent.

Sheep will continuously inhabit the large Gunpowder Neck pen; samples of sheep blood, caged white perch, soil, water, and air will be taken from this area 24 hours before and 24 hours after each lethal agent test. If results indicate that contamination has occurred, sampling schedules will be modified appropriately.

Sampling schedules will generally vary with season and meteorological conditions. If, for example, precipitation exceeds ¼ inch in any 24-hour period, soil and water samples will be obtained. If the estuary surrounding Carroll Island is frozen or natural vegetation is not available in sheep pens for forage, sampling schedules or testing schedules will be modified accordingly.

Starlings will be shot in control (uncontaminated) areas on Carroll Island on 1 day each quarter for brain ChE measurements. These results will be compared with brain ChE measurements on starlings obtained from control areas on other parts of Edgewood Arsenal.

C. ANALYSES. (Investigations to develop and modify analytical methods were conducted by Samuel Sass and Abraham Koblin.)

Methods for measuring ChE in various tissues from the indicator species were discussed in chapter 6.

Two air samplers (beaded bubblers charged with 5 ml of hexylene glycol; flow rate = 1 liter/min) will be located at each soil and water station at heights of 5 feet. This sampling will augment routine vertical and horizontal air sampling conducted around the test grid. Anticholinesterase activity in each sample will be measured using an enzyme inhibition technique.

During FY70 equipment was designed, and methods were selected and modified to extract, qualitate, and measure lethal agent residues, degradation products, and metabolites in samples of soil, water, and vegetation from Carroll Island. Several methods of extracting agent residues from grass and soil were studied. In all methods, samples were extracted sequentially, with the choice of solvent depending on the agent in question. G agents, for example, were extracted with hexane, then chloroform, and finally with acidic (0.01 N HCl) methanol. With V agents, acidic methanol, isopropanol, and chloroform were used. Extracts were concentrated and examined using gas and thin-layer chromatography, colorimetry, and infrared and mass spectrometry. When intact agent or a physiologically active residue was suspected, an enzyme inhibition technique was also employed.

Carroll Island water samples were extracted first with hexanc, then with aninydrous diethyl ether in a continuous extraction apparatus. The hexane and ether fractions were reduced, under vacuum, to near dryness and analyzed for agent or degradation products.

The bulk of the water analyses was done using gas chromatography in conjunction with specific detectors (electron capture, flame ionization, specific phosphorus, and total ion monitor). Materials evident on total ion monitor were subjected to further analysis using mass spectroscopy. Other methods variously employed in examining the hexane and ether extracts included thin-layer chromatography, UV spectroscopy, and infrared spectroscopy.

During the investigations, several impurities were found in the extracting solvents that could produce erroneous results if their presence were not suspected. Current investigations will identify these interfering compounds and will assess the analytical error they produce.

D. BASELINE DATA. (Sheep ChE studies were conducted by 1LT George D. Edwards; residue studies were conducted by Abraham Koblin and Robert T. Howe.)

No lethal agent tests occurred at Carroll Island after 14 July 1969. During the remaining 11½ months in FY70, investigators accumulated pertinent baseline data on indicator species, selected and modified extraction and analytical techniques, and analyzed soil, vegetation, and water samples from the test area to measure residual contamination.

Blood samples were taken from the Carroll Island sentry sheep six times during FY70. Slight variations in RBC and plasma ChE activity were believed to be related to stress of handling and transportation, changes in diet, or normal variation of ChE.

Water samples were collected at eight stations* on 3 November 1969; pH, sediments, dissolved solids, chlorides, and phosphates were measured in each. The lethal agent content of each sample, equivalent to VX, was less than 0.0025 ppin as measured by enzymatic inhibition. This value appears to be a reasonable baseline for future reference; because of limits of analytical sensitivity, this level is not necessarily due to actual agent.

Another set of eight water samples was obtained from Carroll Island, and different concentrations of VX were prepared from each. The stability of the agent was inversely proportional to the pH; ¹⁶ i.e., the more alkaline the water sample, the less VX remaining at the end of the 28-day observation period. Results of periodic analyses also indicated that degradation rates of VX were independent of original concentration, i.e., half lives of high and low concentrations of VX in aliquots of the same water sample were similar. In all but the most alkaline sample, the half life of VX was 10 to 14 days; in the most alkaline sample (pH 7.91, from the sump pump) VX half life was 7 days. The half life of two organophosphate degradation products of VX in water was less than 24 hours, even when the pH was adjusted to 5.8.

^{*}The ninth water sampling station (seventh fish exposure cage) at Hawthorne Cove was added after these initial water samples were collected (appendix).

¹⁶Epstein, J. Nerve Gas in Public Water. Pub. Health Rep. 71 (10), 955-962 (1956).

CHAPTER 8

TOXICITY STUDIES

Edmund J. Owens, John T. Weimer, Paul F. Robinson, and Dr. Elmer G. Worthley directed various studies in this portion of the program.

A. ESTUARINE SPECIES.

1. Toxicity of VX to White Perch, Striped Bass, and Crabs. (Principal investigators were John T. Weimer and Edmund J. Owens.¹⁷)

Three estuarine species were exposed to various solutions of VX and GD in water samples taken from the Gunpowder River near Carroll Island. The concentration of VX necessary to kill 50% of the following groups in 1 hour was 215 ppm for adult intermolt blue crabs, 0.1 ppm for fingerling striped bass, and 0.085 ppm for small white perch (5 to 6 inches); 0.0057 ppm of GD killed 50% of the white perch (5 to 6 inches) in 1 hour. Table X1 gives additional LC50 values at different time intervals. Figure 16 is a plot of the concentration of agent against time to death; these data indicate that adult intermolt blue crabs should not die if VX concentration is less than about 28 ppm. Also, all white perch under the conditions of the experiment should survive GD concentrations less than 0.004 ppm, and white perch and striped bass should survive concentrations of VX less than 0.03 ppm.

2. Toxicity of Anticholinesterases to Organisms Comprising an Estuarine Food Web. (The principal investigator was Paul F. Robinson.)

Estuarine organisms that are indigenous to the Carroll Island area and that can be easily obtained and maintained or propagated in the laboratory were selected. These included: protozoa (Paramecium multimicronucleatum); copepods (Cyclops sp.); amphipods (Gammarus sp.); ostracods or seed shrimp (Cypridopsis sp.); eubranchiopods or brine shrimp (Artemia salina); mosquito larvae (Culex pipiens and Anopheles quadrimaculatus); and killifish (Fundulus heteroclitus and F. diaphanus).

Amphipods and killifish were most sensitive to aqueous solutions of VX; the protozoa and brine shrimp were not affected by relatively high concentrations of VX for up to 24 hours. Third instar mosquito larvae were more resistant to VX than second-stage larvae.

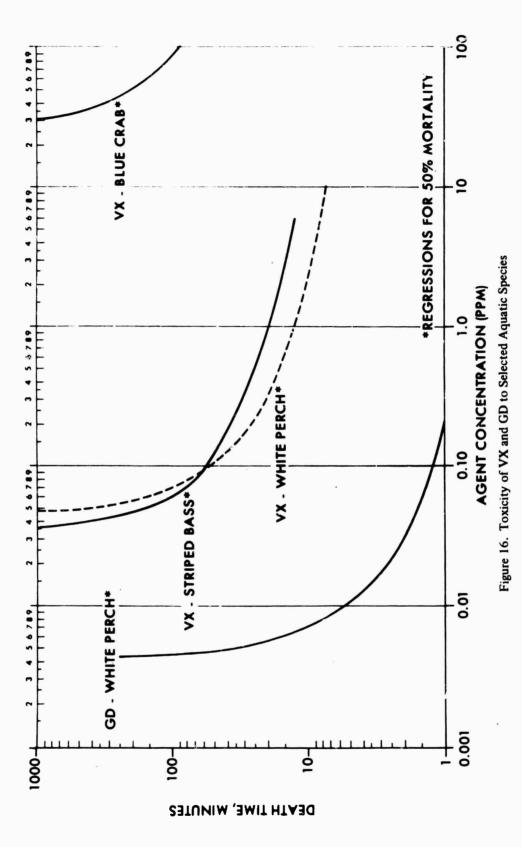
Two degradation products of VX were submitted to Mr. Robinson for toxicological study: the first was not soluble in water; the second product caused incoordination, then death in amphipods in 3 hours at 100 ppm.

Preliminary studies also were conducted on a carbamate insecticide* to provide a basis for comparison with anticholinesterase agents. Amphipods, oligochaetes, and snails (*Physa* sp.) were exposed to various aqueous solutions of the insecticide; again, amphipods were the most sensitive.

These investigations should be completed and a report published during 1971.

¹⁷Weinier, J. T., Owens, F. J., Samuel, J. B., Olson, J. S., and Merkey, R. P. EATR 4441. Toxicity of VX and GD in Carroll Island Test Water Assayed in Indigenous Aquatic Animals. September 1970. UNCLASSIFIED Report.

*Sevin^R—Carbaryl (1-napl hyl-N-methylcarbamate), Union Carbide Corporation, New York, New York.



Harmonic mean statistical analysis.

Table XI. Antichclinesterase Agent Concentrations in Carroll Island Water Necessary to Kill 50% of Three Aquatic Species*

Eugana dia a		GD LC50		
Exposure time	Blue crab	White perch	Striped bass	White perch
hr		į.	рт	
1	215	0.085	0.10	0.0057
2	77	0.062	0.058	0.0055
4	48	0.052	0.044	_
8	36	0.048	0.039	_
16	32	0.047	0.036	_
24	29	0.046	0.035	_

^{*}Based on harmonic mean statistical analysis.

B. PHYTOTOXICITY. (Investigations were conducted by Dr. Elmer G. Worthley¹⁸ and C. Donald Schott.)

The roots of nine species of adult plants and three species of seeds and 7-day-old seedlings were continuously exposed to aqueous solutions of VX of 10, 25, 50, 100, 1000, and 10,600 ppm. The mature plants used in the study were oats (Avena sativa), wheat (Triticum restivum), canna (Canna generalis), coleus (Coleus blumei), petunia (Petunia hybridia), wandering jew (Zebrina pendula), arrowleaf (Maranta bicolor), liverwort (Riccia fluitans), and Eurasian water milfoil (Myriophyllum spicatum).

At an aqueous concentration of 1000 ppm, VX was toxic to all mature plants studied. Coleus plants died in less than 2 weeks at an exposure level of only 10 ppm. Canna, arrowleaf, and liverwort species were affected by 100 ppm. Toxic effects were first evidenced by wilting, which was followed by the appearance of blue-black areas in green leaves and then by total disintegration of all tissues into a jelly-like mass.

Seeds of silky lespedeza (Lespedeza cuneata), bird's-foot trefoil (Lotus cornicalatus), and wheat (T. aestivum) germinated normally when continuously exposed to 10,000 ppm VX. Seven-day-old seedlings of the same three species showed toxic effects when exposed to 1000 ppm VX.

Six-day-old seedlings of the same three species were exposed to various concentrations of malathion, carbaryl, DDT, aldrin, and diazinon. All seedlings showed toxic effects of all pesticides at 1000 and 10,000 ppm after 12 days of continuous exposure. Aldrin was the least toxic, causing only leaf yellowing in seedlings at 1000 ppm.

¹⁸Worthley, E. G. EATF 100-9. The Toxicity of VX to Various Plants. June 1970. UNCLASSIFIED Report.

CHAPTER 9

BASIC ECOLOGICAL INVESTIGATIONS

Dr. Elmer G. Worthley, SP4 Jerry C. Smrchek, 1LT James F. Roelle, SP5 Roy S. Slack, SP4 Alien B. Sheldon, SP4 Harley J. Speir, and Dr. F. P. Ward conducted studies in this portion of the program.

A. TERRESTRIAL STUDIES.

The basic ecological studies discussed in this chapter were designed to implement Bennett Committee remmendation 5 (conduct ecological surveys and an annual census of wildlife). We are attempting to compile a complete inventory of the flora and fauna of Carroll Island and the surrounding estuary. From these preliminary lists, we have selected ecological dominants, representing several trophic levels, that can be relatively easily and reliably inventoried.

These investigations, by name, are basic and thus should indicate any environmental stress (this is in contrast to our monitoring efforts, which have been evolved to detect specifically the presence of lethal agents).

1. Vegetation of Sheep Exposure Pens. (Dr. Elmer G. Worthley was principal investigator.)

In October and November 1969, vegetative surveys of Carroll Island sheep pens and the Gunpowder Neck sheep pen were conducted, and the dominant plant species were tabulated. Results should be published in 1971.

2. Limnological Studies. (SP4 Jerry C. Smrchek was principal investigator.)

Figure 17 is a map of ecological sampling and study areas on and around Carroll Island. Pond 1 was the primary focus of limnological study for the following two reasons.

- a. The pond is located in an area that should not be contaminated by agent tests on Carroll Island.
- b. A large population of spotted turtles found in the pond in the spring of 1970 became the object of a mark-and-recapture survey; limnological data would supplement this investigation.

Seasonal observations were made on portion size and depth, composition of benthic detritus, physicochemical features of the pond water (temperature, pH, hardness, dissolved oxygen, dissolved carbon dioxide), aquatic vegetation (large plants, algae, a bryzoan), and invertebrates present.

A comparative study is being conducted on a similar pond on Gunpowder Neck; results should be published during next fiscal year.

3. Soil-Litter invertebrates. (SP4 Jerry C. Smrchek conducted this investigation.)

Litter-layer invertebrates were chosen for study for several reasons.

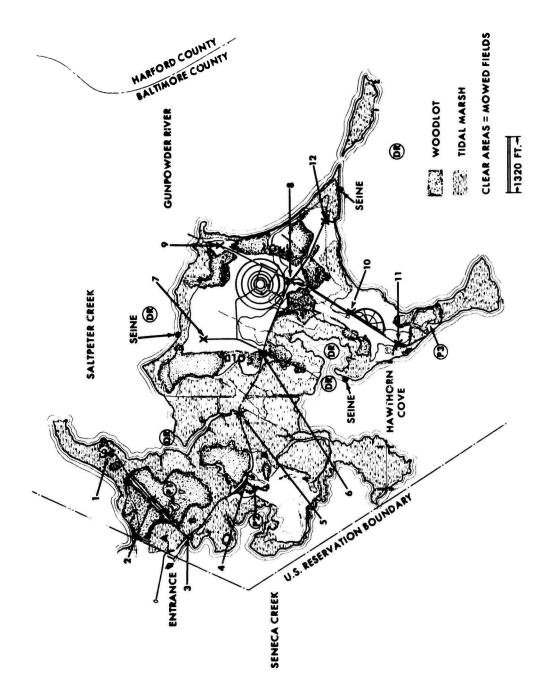


Figure 17. Foci of Ecological Investigations On and Around Carroll Island

Three seining sites are shown: DR represents dredging locations; P1, P2, and P3 are ponds; numbers 1 through 12 indicate songbird and gamebird censusing stations; HOT designates a possibly contaminated woodlot and COLD is a woodlot that should be free of all agent contamination; and A and B are two marshes where muskrat populations were censused.

- a. These tiny animals form several levels of many food chains in the terrestrial community. Dramatic changes in their populations could have profound impact at other trophic levels.
- b. Their limited powers of dispersal would facilitate long-term studies of selected areas.
- c. They live in intimate contact with the litter-soil substrate; thus they are immediately exposed to particle fallout contamination from an agent cloud.

A modification of the Berlese funnel technique¹⁹ for collecting invertebrates was used. Samples were obtained periodically from 14 May to 26 May 1970. Aerial application of an organophosphate insecticide occurred on the evening of 18 May.

In almost all samples, mites were the most abundant invertebrate group, followed usually by collembola (springtails), then either by ants or other insects. Preliminary counts from an uncontaminated (cold) woodlot were generally higher than those from a contaminated (hot) woodlot; this difference was significant in the counts of springtails. The aerial application of insecticide apparently caused population declines in all groups. This deviation, superimposed on normal population fluctuations, may have contributed to the lack of statistically significant differences in the other groups.

We believe the lower counts in the hot woodlot may be a result of:

- a. Residual lethal agent contamination.
- b. Continuous contamination of the area by other agents, primarily riot control agents.
- c. Habitat differences (the soil types of the two woodlots, for example, are different-figure 3, chapter 5).

The approach of this preliminary investigation has been modified, and samples are now obtained biweekly from twelve 1-sq m plots, three each in the hot and cold woodlots and three each in the mowed fields adjacent to the two woodlots.

4. Inventory of Carroll Island Vertebrates. (1LT James E. Roelle was the principal investigator.)

A species list of vertebrates identified on Carroll Island during FY70 includes 9 species of mammals, 123 species of birds, 12 species of reptiles, and 7 species of amphibians. Probable additions to the inventory during the next year will be many species of migrating birds. A list of fish of the Carroll Island area is also being compiled.

5. Turtle Surveys. (SP4 Allen B. Sheldon and SP4 Harley J. Speir were coinvestigators.)

Turtle surveys fit well into the type of ecology program we are conducting on the Carroll Island test area because:

a. Many species of turtles are abundant on Carroll Island.

¹⁹Berlese, A. Apparecchio per Raccogliere Presto ed in Gran Numero Piccoli. Artropod. Redia 2, 85-89 (1905).

- b. Their distribution is islandwide.
- c. They are long-lived; thus, individuals could be studied for many years.
- d. Dispersal of most species, especially some pond-dwellers, is limited.

In April 1970 we began to collect, mark, and release turtles. This would permit us eventually to calculate population density, age distribution, natality and mortality, and movement and growth of individuals.

Turtles have been captured in ponds 1, 2, and 3 (figure 17) and on most roads on the island. Many were hand-caught from the ponds on warm, sunny mornings early in spring. Many females were caught on roads later in the spring as they migrated to find nesting sites. Pond 2 is shallow and small, and many turtles were found here by groping with h. nds and feet.

An entry was made for each turtle; data on species, sex, weight, age, length and width of carapace, distinguishing features (unusual color, broken scutes, missing appendages), and date and place captured were recorded. Turtles were individually numbered by filing combinations of notches in marginal scutes. They were released where captured; all recaptures were recorded, but new measurements and weights will not be taken until next year.

As of 30 June 1970, 201 turtles of five species (48 mud turtles, 101 spotted turtles, 31 painted turtles, 12 box turtles, and 9 snapping turtles) had been marked and released. Numbers of recaptures are at present too low to permit statistical estimates of population sizes.

Intensive capture efforts next spring should provide sufficient data to calculate ecological parameters, including individual growth rates, and population characteristics.

6. Songbird and Gamebird Censuses. (SP5 Roy S. Slack and 1LT James E. Roelle were coinvestigators.)

a. Breeding Songbirds.

Songbirds were chosen for study because of their abundance, their tendency to stay in a restricted area, and their vigorous and obvious defense of territory. Twelve stations, at ¼-mile intervals, were selected near landmarks along Carroll Island roads (figure 17). On 8 days in May 1970, the route was driven by two observers beginning ½ hour before sunrise. Rainy days or mornings with high winds were avoided because these conditions inhibit song activity. The observers identified and counted all birds in 3 minutes at each station. The most abundant songsters were yellow-throats and Carolina wrens. Results will be published during next fiscal year.

On 20 May, Chandler Robbins (author of *Birds of North America*, Golden Press, New York, New York, 1966) visited Carroll Island and conducted an independent breeding songbird count with our two observers. The results were in close agreement.

b. Gamebirds.

Two types of indices, call counts and sightings, were calculated for spring populations of quail and pheasants. Bobwhite quail were indexed in the manner described for songbirds, and pheasants were counted using a crowing cock count (number of crows heard in 2 minutes at each of the 12 stops).

Both species were also indexed by marking the locations of all individuals seen or heard in I day on a transparent map of the island. By superimposing these maps, the investigators obtained an index to the individuals present by clumping observations.

Maps of locations of quail sighting indicated the presence of at least 13 territorial males. The pheasant crowing cock count yielded an average of 13.6 crows per route, and maps indicated the presence of at least 13 pairs.

Carroll Island appears to have an abundant and fairly diversified avifauna; on the day of Chandler Robbins' visit, for example, 85 species of birds were sighted in about 6 hours.

7. Mammalian Censuses.

Dr. Vagn Flyger, Chairman, Department of Inland Research, Natural Resources Institute, University of Maryland, was consulted to aid in selecting species and techniques for mammalian censuses. Dr. Flyger visited Carroll Island and suggested that we could easily count several species, including deer, rabbits, muskrats, and small mammals.

a. Deer. (SP5 Roy S. Slack conducted this study.)

Twenty-two white-tailed deer were counted on Carroll Island on 8 January 1970. Four to six inches of snow had fallen the previous day and provided an excellent background for the helicopter count. Five small herds were seen in the various woodlots.

b. Rabbits. (SP5 Roy S. Slack and SP4 Jerry C. Smrchek were coinvestigators.)

Cottontail rabbits were captured and marked on Carroll Island during the late winter months. Earl Hodil of the Maryland Department of Game and Inland Fish visited the test area on 20 January 1970 and supervised the placement of 200 box-type rabbit traps. Traps were placed randomly in the hot and cold woodlots (figure 17) and were baited with apple slices. The traps were checked daily from 20 to 30 January (rebaited as necessary) and were not operated again until a recapture period from 17 to 28 February. Each captured rabbit was numbered with an aluminum ear tag and released. Recaptures were recorded, and population indices for both woodlots were calculated using the Lincoln Index, ²⁰ Hartley's Method of the Frequency of Recapture, ²¹ and a trap-night index. Results are shown in table XII.

A spring cottontail index of abundance was obtained by counting the rabbits observed per songbird route on 6 days in May. An average of 5.7 rabbits was seen per trip.

c. Muskrats. (1LT James E. Roelle and SP5 Roy S. Slack conducted this study.)

In early April 1970 the investigators attempted to devise a satisfactory census technique for muskrats. Two marshes (A and B in figure 17) were chosen because they were accessible, relatively small, had well-define boundaries, and were in a noncontaminated area closest to the private land adjacent to Carroll Island. The investigators selected 14 muskrat lodges in marsh A and dug a hole through the side of each into the living chamber. When examined 1 week later, seven of the defects had been completely repaired. They concluded that the method would be useful for establishing an active-lodge index.

²⁰Lincoln, F. C. Calculating Waterfowl Abundance on the Basis of Banding Returns. US Department of Agriculture Circular 118, 1930.

²¹Hartley, H. O. Maximum Likelihood Estimation from Incomplete Data. Biometrics 14 (2), 174-194 (1958).

Table XII. Population Estimates^a of Cottontail Rabbits, Carroll Island, January to February 1970

Method of calculation	Cold woodlot	Hot woodlo1	Both woodlots
Lincoln index ^b Hartley's frequency	10	10	20
method ^b	12 1	11.3	19.4
Trap-night index ^c	800.0	0.004	0.006

^aSP5 Roy S. Slack and SP4 Jerry C. Smrchek.

On 16 April, 67 lodges in marsh A and 8 lodges in marsh B were disturbed. One week later, 38 of the 67 in marsh A (57%) and 8 of 8 in marsh B were repaired.

By lodge count alone (67 versus 8), one would assume that marsh A had a much larger muskrat population than B. This probably is not entirely true. Dozier² indicated that not all lodges are living quarters; one or more small feeding huts may be associated with each dwelling lodge. A marsh in New York, for example, had 140 feeding units and 52 nesting houses.

Because of size, all 8 huts in marsh B were probably dwelling lodges, but many of the 67 units in marsh A were probably feeding huts. Because this house size difference is now known, it will be considered in subsequent annual muskrat censuses.

d. Small Mammal Censuses. (SP5 Roy S. Slack, 1LT James E. Roelle, and SP4 Jerry C. Smrchek conducted this study.)

Trap-night indices for abundance of small mammal populations were obtained in early May 1970 by trapping two areas, one each in the hot and cold woodlots (figure 17). The trapping areas were approximately 100 feet square and contained 100 small mammal box traps set at 10-foot intervals. Traps were baited with peanut butter and were checked daily for 2 weeks. Captured animals were toe-clipped for identification and released.

The results were disappointing; only one white-footed mouse (*Peromyscus leucopus*) was captured in the cold woodlot, and none were caught in the hot woodlot. The period for trapping should have been during cold months, but traps only became available to us in early May. This study will be repeated during the winter of 1970-71.

B. ESTUARINE STUDIES.

Investigations designed to measure total acute and long-term effects, if any, of chemical agent testing on the estuarine ecosystem surrounding Carroll Island have been initiated.

1. Beach Seining. (SP4 Harley J. Speir was principal investigator.)

In May 1970 a seining study of fish was initiated in waters adjacent to three beaches (figure 17) on Carroll Island. One site could receive surface-water runoff from test areas; a second is in the path of most agent clouds; and a third, a control beach, should not be contaminated.

^bNumbers represent estimated number of rabbits in designated area.

^cIndex equals number of catches per trap per nighl.

²²Dozier, H. L. Estimating Muskrat Populations by House Counts. Trans. 13th N. Amer. Wildlife Conf. 1948, 372-392.

Joseph V. Boone, Maryland Department of Natural Resources, visited Carroll Island several times during FY70, instructing our investigators in technique, methods of recording data, and identification of species. Insuring that our methods are similar to those used by State of Maryland personnel will permit direct comparisons of our data with data accumulated on a statewide basis.

Using a 100- by 4-foot seine, water offshore from the three areas were sampled at least biweekly when no ice was present. Two hauls were made at each site, and all fish and other organisms were identified and counted. With adult fish, a total-length range was recorded for each species; for young-of-year, the mean size of a sample of individuals was recorded. Observations also were made on water temperature, salinity, dissolved oxygen and carbon dioxide, pH, light penetration, tide, bottom composition, vegetation, and invertebrates. Results will be presented in a later report.

2. Studies of the Benthic Community.

Because particulate agents or simulants may sink directly to the bottom, or because many liquid agents in aqueous media adsorb readily to particles, investigators are especially interested in the estuarine sediments surrounding Carroll Island and the benthic organisms living in contact with these sediments.

Bottom samples were obtained, using an Ekman dredge, from five sites surrounding Carroll Island (figure 17) on a weekly basis. Organisms were collected by washing the sediment through sieves; they were preserved in 10% formalin pending identification. Complete results of this study will be presented in a later report.

Preliminary counts of benthic fauna indicated that Carroll Island waters were included in the tremendous Atlantic Coast resurgence of the brackish-water clam Rangia cuneata.²³ Dense beds of young-of-year clams were found surrounding Carroll Island, and beds of the adult mollusks (shell length 40 to 45 mm) were located near the eastern side of Gunpowder Neck.

3. Diamondback Terrapins.

On 29 May 1970, two diamondback terrapins (Malaclemys terrapin terrapin) were caught in a crab pot baited with herring; the pot was set just southeast of Carroll Point and was being used as a source of crabs for laboratory studies. One turtle was alive, but the other had drowned.

On 3 June the seining team reported seeing about 15 diamondbacks swimming in Hawthorne Cove; two terrapins were caught in one of the seine hauls. On 7 June two fishermen on Gunpowder Neck, using pieces of blue crab muscle as bait, hooked seven diamondbacks in a few hours; they reported seeing many more.

Dr. George Zug, Associate Curator, Reptiles and Amphibians Division, Smithsonian Institution, confirmed that these captures were the first scientific records for *M. t. terrapin* in Baltimore County or Harford County. Ten diamondbacks were marked with numbered stainless steel tags requesting the finder to telephone our unit.

Feces from all captured terrapins and the intestinal contents of one turtle found dead contained a high percentage of crushed and broken shells of Rangia cuneata.

²³Hopkins, S. H., and Andrews, J. D. Rangia cuneata on the East Coast: Thousand Mile Range Extension, or Resurgence? Science 167, 868 (1970).

- 4. Fish Kill Caused by Simulant Agent.* (Dr. F. Prescott Ward was principal investigator.)
- On 2 May 1970 a civilian guard reported dead white perch on a beach on Maxwell Point, Gunpowder Neck. Talcum, a simulant agent for CS, was concluded to be the cause of the fish deaths for the following reasons.
- a. The day preceding the kill, 2000 pounds of talc were dispersed in the Gunpowder River adjacent to the site of the incident.
- b. High winds had churned water, mud, and talc into a turbid slurry, and a large quantity of talc had been deposited on the beach at the high tide mark. This talc line extended for about 2800 feet; dead fish (a total of approximately 3000 to 5000) were found only in association with this line of white powder.
- c. On microscopic examination of the gills, crystalline material that was indistinguishable from talc was found lodged between the gill filaments.
- d. Dead fish otherwise appeared normal on gross and histopathologic examinations. Brain cholinesterase was normal. No bacteria or fungi known to be pathogenic to fish could be cultured from any organs.

The test involving the talc was designed to check the area coverage pattern when talc was released from a disperser mounted on a helicopter; the water afforded a uniform background for pattern photographs. Since this incident, pertinent SOP's have been revised to prohibit any over-water releases of simulant agents.

^{*}Edgewood Arsenal Technical Memorandum 100-14 entitled, "Maxwell Point White Perch Kill, 2 May 1970; Talcum Identified as Cause" (F. P. Ward, S. Sass, D. G. Fairchild, L. L. Kunz, R. A. Renne, R. J. Piffath, and T. L. Fisher) is in zeess.

CHAPTER 10

CONTINUING RESEARCH

Additional efforts during FY71 will be in the following fields.

A. COMPUTER USE.

Our computer needs are threefold:

- 1. Routine statistical treatment of data, e.g., least squares regressions; tests of significance; and computations of means, standard deviations, and other statistical parameters.
- 2. Data storage and retrieval. The volume of information generated by the various segments of this program is nearing the point where one loses familiarity with the data. We have almost lost louch with observations and results that would guide and channel future research.
- 3. Systems analysis. We hope eventually to have gleaned and programmed sufficient information on the ecological effects of chemical agents to be able to predict their environmental impact in selected ecosystems.

Perhaps the greatest current problem in our recording of data is caused by the high manpower turnover in the program; after several personnel changes, stored data can become lost in individual notebooks. We have attempted to circumvent this problem by using uniform recordkeeping procedures, but field notes and many ther observations are impossible to standardize.

1LT James E. Roelle made preliminary inquiries regarding computer use in our program. He conferred with statisticians assigned to the Operations Research Group and the Office of the Comptroller on Edgewood Arsenal. He also visited the Bird Banding Laboratory, Migratory Bird Populations Station, Fish and Wildlife Service, US Department of the Interior; this agency administers the huge North American bird banding program and is supported by a sophisticated electronic data-processing unit. He also plans a visit to the Smithsonian Institution to confer with data-processing personnel.

During FY71 we intend that all data will be entered on punch cards, and several equations (population interactions, mortality and natality rates, population growth forms) will be programmed.

B. SURVEYS AND CENSUSES.

Several studies within this segment of the program will receive increased emphasis.

1. Estuarine Research.

Our fisheries biologist and limnologist have been appointed as principal investigators on a research task to ascertain the effects of chemical agents on the estuarine ecosystem surrounding Carroll Island. Basic information will be accumulated on the following:

a. Normal flora and fauna of the area, with ecological dominants delineated.

- b. Assessments of limiting factors including measurement of water temperature, salinity, dissolved oxygen, biogenic salts and other nutrients, bottom composition, depths, tidal currents, tidal extremes, and light penetration.
 - c. Descriptions of communities (benthic, periphytic, planktonic).
 - d. Intra- and interspecific population interactions.
- e. Estimations of natality, mortality, and dispersal (particular emphasis on anadromous species of fish) and assessments of periodicities (especially seasonal, tidal, and daily).
- f. Descriptions of atural food webs and measurements of energy flow at trophic levels.

The goals of applied research in this area will be:

- a. To measure contamination, if any, of the sediments with chemical agents or simulants.
- b. To establish toxicity of chemical agents to additional estuarine organisms, primarily ecological dominants.
- c. To study the effects of aquatic physicochemical factors on the fate of chemical agents.
- d. To measure propensities of chemical agents to diffuse, adsorb, or settle in different aquatic environments.
- e. To study the local ecological significance of the current population resurgence of the clam, *Rangia cuneata*, and to ascertain whether this filter feeder concentrates any chemical agents in its tissues.

2. Vegetative Survey.

An investigation of Carroll Island flora has been initiated by a botanist, an agronomist, and a wildlife ecologist participating in the program. Research will include:

- a. An inventory of the flora.
- b. Species compositions (frequency, abundance, and cover) and dominants of difference areas.
 - c. Primary productivity of several areas.
- d. Continuing programs on effects of chemical agents on plants and effects of plants on chemical agents.

3. Other Surveys.

Basic information is needed on insects, decomposers, and algae in the Carroll Island ecosystems. We recognize these needs and wil! initiate investigations that fit within the framework of our manpower, expertise, and funds.

LITERATURE CITED

- 1. Chemical Warfare Board publication. Improvements. Carroll's Island. Grace's Quarters. 1944. UNCLASSIFIED Report.
- 2. Van Kampen, K. R., Shupe, J. L., Johnson, A. E., James, L. F., Smart, R. A., and Rasmussen, J. E. Effects of Nerve Gas Poisoning in Sheep in Skull Valley, Utah. J. Amer. Vet. Med. Ass. 156 (8) 1032-1035 (1970).
- 3. Report of the Ad Hoc Advisory Committee for Review of Testing Safety at Edgewood Arsenal, Maryland and Fort McClellan, Alabama. Department of the Army, Washington, D.C. September 1969. UNCLASSIFIED Report.
- 4. Soil Survey Interpretations for Baltimore County, Maryland. US Department of Agriculture, Soil Conservation Service, Cockeysville, Maryland. April 1966.
- 5. Harlin, B. W. Final Report. Technical Report ECOM 6039. The Edgewood Arsenal Climate Calendar. Environmental Science Services Administration, US Army Electronics Command, Meteorological Support Activity, Fort Huachuca, Arizona. April 1969. UNCLASSIFIED Report.
- 6. Englebrecht, H. H. Climates of the States. Maryland. Climatography of the United States No. 60-18. Weather Bureau, US Department of Commerce, Washington, D.C. December 1959.
- 7. US Army Meteorological Team Data, Edgewood Arsenal, Maryland. Monthly Reports, July 1968 to October 1969, inclusive. Meteorological Support Activity, US Army Electronics Command, Fort Huachuca, Arizona. 16 documents. UNCLASSIFIED Reports.
- 8. EA Pamphiet 70-2. Research and Development. Carroll Island Agent Testing Complex. 26 January 1970. UNCLASSIFIED Report.
- 9. Military Chemistry and Chemical Agents. Department of the Army Technical Manual 3-215 (December 1963), with Change No. 1 (16 March 1965), and Change No. 2 (15 June 1967). Department of the Army, Washington, D.C. 1-51.
- 10. TB MED 282. Anticholinesterase Intoxication: Pathophysiology, Signs and Symptoms and Management. Department of the Army, Washington, D.C. 21 January 1970. UNCLASSIFIED Report.
- 11. TSDSOP No. 4-2-69-17. Open Air Testing of Lethal, Incapacitating and Riot Control Agents at Edgewood Arsenal. Submitted 14 November 1969. UNCLASSIFIED Report.
- 12. EA Regulation 10-1. Organization and Management Manual. 7 July 1966. UNCLASSIFIED Report.
 - 13. EA Regulation 385-3. Safety. Range Areas. 3 April 1968. UNCLASSIFIED Report.
- 14. Ward. F. P., and Hess, T. L. Automated Cholinesterase Measurements: Canine Erythrocytes and Plasma. Amer. J. Vet. Res. 32 (3):499-503 (1971).

- 15. Pyle, R., and Cronin, E. The General Anatomy of the Blue Crab. Callinectes sapidus Rathbun. State of Maryland, Board of Natural Resources, Department of Research and Education. Chesapeake Biological Laboratory Publication 87. August 1950.
 - 16. Epstein, J. Nerve Gas in Public Water. Pub. Health Rep. 71 (10), 955-962 (1956).
- 17. Weimer, J. T., Owens, E. J., Samuel, J. B., Olson, J. S., and Merkey, R. P. EATR 4441. Toxicity of VX and GD in Carroll Island Test Water Assayed in Indigenous Aquatic Animals. September 1970. UNCLASSIFIED Report.
- 18. Worthley, E. G. EATM 100-9. The Toxicity of VX to Various Plants. June 1970. UNCLASSIFIED Report.
- 19. Berlese, A. Apparecchio per Raccogliere Presto ed in Gran Numero Piccoli. Artropod. Redia 2, 85-89 (1905).
- 20. Lincoln, F. C. Calculating Waterfowl Abundance on the Basis of Banding Returns. US Department of Agriculture Circular 118, 1930.
- 21. Hartley, H. O. Maximum Likelihood Estimation from Incomplete Data. Biometrics 14 (2), 174-194 (1958).
- 22. Dozier, H. L. Estimating Muskrat Populations by House Counts. Trans. 13th N. Amer. Wildlife Conf. 1948, 372-392.
- 23. Hopkins, S. H., and Andrews, J. D. Rangia cuneata on the East Coast: Thousand Mile Range Extension, or Resurgence? Science 167, 868 (1970).

APPENDIX

LOCATIONS OF CARROLL ISLAND MONITOR STATIONS

A. LAND STATIONS.

Sheep pens and soil and air sampling:

- 1. Sheep holding pen-Located directly behind the Carroll Island office, 1520 yards west of the center of Agent Test Grid 1
- 2. CP pen-Located directly across Carroll Island road from Control Point, 560 yards west of grid center
- 3. Burning pit pen-Near the northwest corner of the Spray Grid, 640 yards NNW of grid center
 - 4. Magazine area pen-440 yards NNE of grid center
 - 5. Hot area pen-330 yards ESE of grid center
 - 6. Grid 2 pen-885 yards south of grid center
- 7. Gunpowder Neck pen-Located at Gun Position 2 on Gunpowder Neck, 4300 yards east of grid center

B. LAND STATION.

Soil and air sampling only:

Contaminated sample taken between 30- and 40-yard sampling circles of Test Grid 1, downwind from each lethal agent test.

C. WATER STATIONS.

Fish exposure cages and air and water sampling:

- 1. Dock—Area located at the end of Carroll Island dock, 750 yards NNE of grid center.
- 2. Gunpowder River, north of Carroll Point-1200 yards east of grid center; 100 yards offshore and on a line from wind tunnel exhaust stack to tallest tree in grove on north side of chamber.
- 3. Chesapeake Bay, south of Carroll Point—In Bay Cove 1200 yards SE of grid center; 100 yards offshore and south of wind tunnel on a line from exhaust stack of wind tunnel to tallest tree in grove on north side of chamber. Distance offshore on a line between end of trees on Carroll Point and building near Agent Test Grid 2.
- 4. Hawthorne Creek inlet, 650 yards SSW of grid center in midchannel at the cable crossing.

- 5. Hawthorne Cove, 1000 yards SSW of grid center on a line from Brier Point to end of road south of Grid 2.
- 6. Seneca Creek control, 2200 yards west of grid center; in midchannel next to hydroelectric plant and on a line between point of land on Carroll Island and barge.
- 7. Gunpowder River, 100 yards offshore from Gun Position 2 and on a line between gun mount and end of tree line on Carroll Point, 4100 yards east of grid center.

D. WATER STATIONS.

Water and air sampling only:

- 1. Sump pump under Agent Test Grid 1 (no air sampler).
- 2. Drainage ditch on north side of Carroll Island road, 300 yards WSW of grid center.